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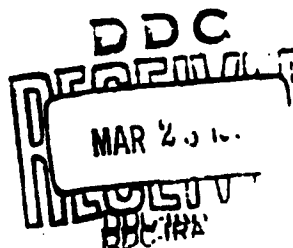
P.F. ZDRODOVSKII

TYPHUS FEVER AND BRILL'S DISEASE  
(Etiology, pathology, epidemiology and prevention)

Moscow, 1965

NOTE

The present monograph contains basic data collected from the world literature and gained from original research on the etiology, pathology, immunology, epidemiology, and prevention of classical typhus fever compared with sporadic typhus, its recurrent form which corresponds to BRILL's disease.



PREFACE

The book to which we call the readers' attention gives a comprehensive account of the typhus fever problem in its more important aspects in accordance with the modern knowledge of this infection. During the past twenty years this knowledge was enriched with many new findings due to the elaboration and wide practical appropriation of new microbiological and sero-immunological methods in rickettsiology. The new findings entailed correction of some previous concepts about the nature of residual forms of typhus fever labelled as sporadic, or recurrent typhus which do not fit into the established epidemiological concept of classical or epidemic-typhus fever. Due to the wide use of sero-immunological methods, our position was also strengthened in regard to the existence of atypical and subclinical forms of typhus fever infection. The very methods of the epidemiological study of this infection were also changed. At the present time, such a study is no more conceivable without the employment of sero-immunological research methods which, in a previously inaccessible form, permit to retrospectively expose the past of typhus fever and its present status in a surveyed country. Finally, a new large chapter was created for the specific prevention of typhus fever with the aid of various types of vaccines. At present this chapter ends with the appropriation of the most perfect form of antityphus vaccine---the live vaccine which renders a marked and long-lasting immunity already on the next day after a single inoculation.

In addition to the more important data from the world literature which includes a rather valuable contribution of our previous and modern national authors, the book also summarizes the results of many years' work on the typhus fever section of rickettsiases which was directed by the author.

In the book, we carefully used the very valuable typhus-fever observations of specialists in our brother republics---especially in Rumania and Poland, just as also in our country, which acquired a serious typhus inheritance due to the plundering of the Fascist armies on the areas temporarily occupied by them. Just as our brotherly neighbours, today we experience the process of getting rid of the residual forms of this infection in the shape of sporadic or recurrent (second) typhus fever.

In the general balance of sanitary conditions of our country, the specific share of typhus fever in its sporadic form is negligible. But we know well that under unfavorable conditions and in presence of pediculosis this "peaceful" form of typhus fever can become the source of an epidemic typhus. This is why, in spite of the absence of epidemic typhus fever, one must vigilantly watch over this potential menace, by using all measures for its ultimate elimination. All the above said also justifies the appearance of this book, the more so because in our national literature, although there are excellent clinical monographs on typhus fever, there is no monograph of the type offered in the present publication, unless we consider the book of V.A. BARYKIN and I.I. DOBREITSER on "Typhus Fever", published in 1932, which is only of a historical interest. Valuable contributions to the understanding of the nature and epidemiology of sporadic typhus were brought by works from the laboratory of K.N. TOKAREVICH (Leningrad), published in the form of three Collections (Sbornik, 1952; 1958; 1961), and by a series of articles from the laboratory of G.S. MOSING (L'vov). But unfortunately not all these works had a monographic generalization for their authors. With full attention, the present author used the material of these authors in this book.

The author distinctly foresees that the published book may provoke a sharp protest on the part of many domestic epidemiologists who are adversely tuned against the recurrent nature of sporadic typhus fever. But in the science facts decide which accumulated in a continuous movement, and not the protection of shades of the past.

P. ZDRODOVSKII

GENERAL INFORMATION ON TYPHUS FEVER

The synonyms of typhus fever are: epidemic, louse-borne, historical, European typhus fever; sporadic typhus fever, Brill's disease; louse-borne typhus (Engl.); typhus exanthématique (French); Fleckfieber (German - Spotted Fever); typhus exanthematicus, Rickettsiasis epidemica pediculosa (Lat.). [Abbreviated in this monograph to TF].

DEFINITION

Under typhus fever we understand an acute febrile rickettsial ailment of varying severity which runs with marked symptoms of central nervous system toxicosis, in presence of a diffuse roseolar petechial exanthema, with formation of vasculitides and thrombovasculitides in small vessels of various localization and strength. The agent of TF is the Rickettsia prowazeki (henceforth: RPr), and its transmitter is the body louse (and partly the head louse) which becomes infected while sucking the blood of sick people and, dropping upon healthy individuals, propagates the typhus infection through its fecalia which contain rickettsiae, by dropping the latter into small skin wounds (scratches) and on external mucous membrane. In difference from other rickettsiasis, typhus evidently does not have a virus reservoir in external Nature, but, by spreading through lice from man to man, in case of pediculosis of the population and in presence of other advantageous conditions, it can provoke extensive epidemics and pandemics. Hence, the potential epidemic importance of TF is great for public health. Beside the epidemic forms, TF varies considerably, it is the highest among elderly people. The course of sporadic TF is usually benign.

HISTORY

The first clinical epidemiological description of typhus was given in the XVI. century by Girolamo FRACASTORO (1546). However, only in the mid-19th century was TF separated as an independent nosological entity with its differential diagnosis from other "typhous" ailments (recurrent and abdominal typhus). This was the result of the works of GERHARD in the USA (1887), S. P. BOTKIN (1868) in Russia, GRIESINGER (1857) in Germany, and S. MURCHISON (1862) in England. The infectious nature of TF and the presence of its agent in the blood were accurately shown by the Russian investigator O.O. MOCHUTKOVSKY the first time in 1876 in a heroic experiment of self-inoculation of the blood of typhus patients.

In 1878 the Russian investigator G. N. MINKH was the first to announce "the high probability of the transmission of recurrent and exanthematous TF from man to man with the aid of blood-sucking insects". (Quoted after A.I. METELKIN, 1951). Later, the French investigators NETTER and THOINOT, when they analyzed the outbreak of TF in France in 1892-1893, announced in their turn the hypothesis on the propagation of TF by lice. In 1908 the communication of N.F. GAMALEYA appeared in which, on the basis of epidemiological findings, he corroborated that TF could be caught only in the presence of lice. Finally, in 1909 the well-known French investigator Ch. NICOLLE showed in experiments on monkeys that the body louse is the transmitter of TF infection. This is also the basis of modern ideas on the epidemiology of the disease, and it made possible to have a good foundation for the conventional measures in the control of this rickettsiasis. On the basis of epidemiological data, the essence of these measures (control of pediculosis) was also independently provided by N.F. GAMALEYA in the same 1909 year.

Later on, the problem about the pathogenic agent of TF also found its solution. The partially successful preliminary searches after this agent were carried out by the American investigators H. RICKETTS and R. WILDER in Mexico (1909-1910), and by the Czech investigator S. PROWAZEK in Eastern Europe (1913-1914), but the researches were not completed since they were interrupted by the death of both authors who became infected with TF at its study. Continuing the search of S. PROWAZEK by studying the agent of TF in infected lice, the Brazilian investigator H. DA ROCHA-LIMA, while working at that time in the Hamburg Tropical Institute, in 1916 successfully completed

the search of his predecessors, and isolated the agent of TF in the form of a peculiar intracellular microorganism which lives as a parasite in the epithelium of the stomach of infected lice, and is excreted with louse fecalia. In honor of the pioneer researchers H. RICKETTS and S. PROWAZEK, who died at the study of TF, Da ROCHA LIMA published the agent of TF which he differentiated under the name of Rickettsia Prowazeki (RPr), simultaneously emphasizing by this term the specificity of the group of microorganisms to which the agent of TF belongs, which also retained the term "Rickettsia" from this time on.

For the study of TF, the experimental reproduction of rickettsial infection in animals was of very great importance. This task found a solution in its time on the one hand in the works of Ch. NICOLLE and his collaborators (1909-1912), who established the susceptibility of monkeys to TF infection, and on the other hand in the works of GAVINO and GIRARD (1910-1911) as well as NICOLLE and his coworkers (1912) who showed that to the TF agent guinea pigs are susceptible, animals generally accepted at present as models for the reproduction of TF infection under laboratory conditions.

As intracellular parasites the rickettsiae which provoke TF cannot be cultivated on the ordinary nutrient media used by bacteriologists, and the rather important question of their accumulation under laboratory conditions required research of many years. Originally, lice were used for this purpose, for whose infection (with RPr) R. WEIGL suggested microenemas in 1919. This painstaking and low-efficient method of accumulating rickettsias in lice, although it was also greatly perfected in the laboratories of G.S. MOSING (L'vov) during 1941-1942), was replaced by the Soviet authors A. V. PSHENICHNOV and B. I. RAIKHER with a simple method of massive infection of louse larvae by means of feeding them with infected blood through an "epidermal membrane", taken from the skin of human cadavers and later replaced by the American investigators T. SNYDER and WHEELER (1945) with skins of young chicks.

The work on body louse was considerably complicated by the need to feed them by way of blood sucking on donor people. To avoid this difficulty, the American authors H. FULLER, J. SNYDER and E. MURRAY (1949) took lice which were adapted to feed on rabbits, which was later on independently carried out also in the laboratories of A. V. PSHENICHNOV (1957). The employment of the indicated methods of infection through membranes with the use of adapted races of body lice made all the work considerably easier in their cultivation and infection with RPr.

Further on, the French investigators P. DURAND and H. SPARROW, having used the method of the Mexican author M. CASTANEDA (1939), in 1940 published a method which they elaborated for massive accumulation of RPr in the lungs of rodents and especially of mice after their intranasal inoculation.

Finally, in 1938-1940, the American researcher H. COX, who by his own statement was inspired by the researches of V. A. BARYKIN (1937), elaborated the method of massive cultivation of rickettsiae, including also the cultivation of RPr (1940) in the vitelline sacs of chick embryos.

As a result of this, the laboratories were able to cultivate the agent of TF in any amount, by accumulating them in the gastro-intestinal tract of lice, in the lungs of rodents, or in the vitelline sacs of chick embryos, which in its turn assured the possibility to prepare antigens from RPr for the specific serodiagnosis of TF by agglutination and complement fixation reactions, which until that time was limited by the use of a non-specific agglutination of an antigen from Proteus OX<sub>19</sub> culture, proposed in 1916 by E. WEIL and A. FELIX.



The possibility of accumulating RPr in any quantity under laboratory conditions permitted also to solve the problem of specific immuno-prevention of TF with the output of different types of vaccines for mass employment (louse vaccine of WEIGL and PSHENICHNOVRAIKHER, the pulmonary vaccine of DURAND-GIROUD, and the egg vaccine of COX).

Together with the above, we specially mention the researches of D. V. POPOV (1875) who was the first to call attention to the formation of nodules (granules) in the brain characteristic for TF, and the much later works of FRAENKEL (1914) and of N.A. ALFEEVSKY (1914) on the vascular lesions specific for TF as well as the classical research of I.V. DAVYDOVSKY (1921-1922) on the pathological anatomy of TF. Finally, in the understanding of the pathogenesis of TF, the discovery of the toxic properties of RPr played an important role. These properties cause the formation of neutralizing antibodies in the organism (E. GILDEMEISTER, and E. HAAGEN (1940); GIROUD (1938).

In the history of TF research, special attention is also due to the separation of the benign forms of "sporadic" TF, described by N. BRILL the first time in 1910 in the USA, and later on explained by H. ZINSSER (1934) as recurrence of latent forms of an earlier transmitted epidemic (louse-borne) TF. The indicated variants of TF obtained the name of Brill-Zinsser disease in the literature.

In the sphere of treating TF, and treating other rickettsial diseases during the last decade, the use of antibiotics (chloromycetine, derivatives of the tetracycline series), gained exclusive importance, usually assuring a quick and reliable cure of the disease.

In conclusion, we should specially mention the family of our native authors (beside the ones already cited above) who added some kind of valuable contribution to the study of TF. Here, first of all those Russian physicians should be mentioned who were the original pioneers in the study of TF. In particular, these physicians are YA. SHIROVSKII (1811), YA. GOVAROV (1812) and I. FRANK (1825).

Among the later and modern native authors who wrote more important works on the epidemiology and etiology of TF, we mention L.A. TARASEVICH, A.N. SYSIN, I.A. DOBRETTSEV, V.A. BARYKIN, G.V. EPSTEIN, L.V. GROMASHEVSKII, G.S. MOSING, K.N. TOKAREVICH, E.M. GOLINEVICH, E.N. PAVLOVSKII, M.K. KRONTOVSKAYA, M.M. MAEVSKII, I.V. ROGOZIN, and a number of clinicians: K.F. FLEROV, G.A. IVASHENTSEV, N.K. ROZENBERG, D.D. PLETNEV, M.P. KIREEV, N.A. RAGOZA, A.M. SIGAL, A.N. FILIPPOVICH, A.F. BILIBIN, K.M. LOBAN, and others.

These are the more important phases in the history of many years of research and comprehensive understanding of TF.

#### STATISTICS AND DISSEMINATION

Both in the remote and the near past, including the first half of the twentieth century, epidemics of TF repeatedly provoked ravages among the population of Europe, and beyond its boundaries, especially in the periods of wars and different social shocks (hence the disease was characterized by such synonyms in the past as war typhus, hunger typhus). The below quoted statistical data on the most known epidemics selectively illustrate what was said. At the same time they show the tremendous epidemic potential of TF infection when suitable conditions exist for its dissemination.

In the year 1489, at the siege of Grenada, in the Spanish Army 17,000 men died of TF.

In the year 1528, at the siege of Naples, from the French Army 30,000 soldiers died of TF.

During 1576-1577 in Mexico more than two million people perished by TF.

In 1632 during the 30-year war, under Nuremberg in the Swedish Army, 18,000 soldiers died of TF.

Uncountable were the losses by TF which happened in the Napoleonic Wars in 1812. Thus, in Vilno, out of 30,000 French prisoners of war 25,000 men died of TF, and in Danzig out of 36,000 there were 13,000 death cases, and in Torgay 14,000 out of 26,000 men, and in Mainz 30,000 men, and so on.

During 1816-1819, in Ireland, related to the famine, among 6 million inhabitants about 700,000 persons had had TF, and 40,000 of them died.

In the Crimean War of 1854, in the French Army the TF morbidity rate reached 1:100, and 17,000 soldiers died.

In the Russian-Turkish War of 1877-1878, in the Russian Dunai Army, 32450 persons got sick with RF, and 10,000 men died, while in the Caucasian Army 15,660 got sick and 6,500 died.

During the First World War in 1915 in Serbia more than 150,000 men died of TF, and in particular 126 of 400 physicians died of TF.

In Poland, in connection with the First World War and with its sequelae during 1916-1920 514,000 persons have had TF, of whom 56,494 died.

In Tsarist Russia, TF was a common infection which spread over a large area, including the capital city Peterburg and Moscow. According to the data of L.V. GROMASHEVSKII (1947), during the period from 1880 to 1913, the morbidity index by five-year groups varied within 4.5 and 8.5 per 10,000. Especially, during 1905-1911 a total of 724,436 sickness cases, and 49,213 deaths were recorded due to TF. During this period the lowest morbidity rate per year was 45,000 cases of TF with a peak rate of 160,000. TF kept on roughly on the same level during the First World War (1914-1917). After the revolution, during the Civil War and Foreign Intervention, beginning from 1918 on, TF had a sharp rise, having caused in the young Soviet Republic the pandemic of 1919-1922; moreover, at the height of this pandemic in 1919-1920 in the USSR the average index of morbidity rate per 10,000 inhabitants reached respectively 340 and 339, with a peak of 601.8 (Central Agricultural District), and a minimum of 85.8 (Northern district)(Figures quoted after I.A. DOBREITSER).

As some authors suggest, during the 1918-1922 period alone about 10 million people got sick with TF (I.A. DOBREITSER, 1932).

During the Second World War (1941-1945), and the next subsequent years, TF epidemic was observed in North Africa (1942-1944), Iran and Iraq (1943), Southern Italy (1943-1944), Yugoslavia (1944), Rumania (1944-1947), Poland (1940-1946) and others.

Especially, an intensive TF outbreak occurred in 1942 in Egypt where about 40,000 had the sickness with a 14% mortality rate. In 1943 in Iran and Iraq, during the TF epidemic, 3338 patients were hospitalized with a mortality rate between 9.8% and 12%. From December 1943 until February 1944 in Naples 1423 TF cases were recorded with a mortality rate of 13.9%. During 1940-1946 in Central Poland about 220,000 TF sickness cases occurred, while in Rumania during 1944-1947, 143,284 TF patients were recorded.

As it can be seen from the quoted data, TF epidemics were observed during the Second World War.

As to the USSR, during the Second World War (1942-1945), epidemic TF had a spread among the civilian population in the districts temporarily occupied by Fascists. Further on, after the restoration of the country's economy, TF ceased as an epidemic ailment, remaining only in the form of sporadic sicknesses (See Table 1).

Such was the epidemic potential of TF infection in reflection of its past epidemics in the various epochs and in the various countries.

From among the individual regularities which characterize the spread of TF, first of all the morbidity rate by age groups deserves attention. An illustrative presentation of this is Table 1, which was compiled according to long-range findings of the age distribution of TF cases in Moscow.

TABLE 1

TYPHUS FEVER MORBIDITY RATE IN MOSCOW BY AGE GROUPS  
(After I.A. DOBREITSER, 1922)

Age in years	Years	Absolute Figures			Percentage to total number of sickness		
		1916-17	1919-23	1924-28	1916-17	1919-23	1924-28
Δo 1	---	86	---	0.0	0.1	0.0	
1----4	12	741	11	0.8	0.9	0.7	
5----9	33	2782	27	2.1	3.3	1.8	
10---14	104	6124	86	6.7	7.5	5.8	
0----14	149	9683	124	9.6	11.8	8.3	
15---19	375	12414	509	24.9	15.2	13.1	
20---29	395	25861	357	25.7	31.6	34.5	
30---39	306	17442	124	19.9	21.3	24.2	
40---49	228	10721	181	14.8	13.1	12.3	
50---59	600	4421	90	3.9	5.4	6.1	
60	17	1273	22	1.1	1.6	1.5	
TOTAL		1530	81815	1476	100.0	100.0	100.0

As it can be seen from Table 1, the peak of TF affliction concerns the flowering age --20-29-years -- with the contiguous age groups of 15-19 and 30-39 years, while the minimum morbidity rate is found in the children's group up to 9 years of age. These ratios are independent from the intensity of epidemics.

In correspondence with the indicated facts, according to later findings of N.I. RAGOZA (1955), in the period of the Second World War (1941-1945), for the first 20-year age group the morbidity rate was 18.1%, for the second twenty-year group it was 71.5%, i.e., a considerable majority, and, finally, for the third twenty-year group it was only 10.4%, i.e., the minimum.

In regard to TF morbidity rate by sex, all statistics note a slight predominance of the morbidity rate in men in comparison with women. For instance, in Peterburg in 1887-1889 the morbidity rate per 10,000 inhabitants was 31.4 for men and 13.9 for women, while during 1900-1909 it was 20.4, respectively 13.4, and in Petrograd during 1911-1915 it was 0.9 and 0.64 (quoted after I.A. DOBREITSER).

The sex ratios find their reflection also in the comparative TF mortality indices. Thus, according to the data quoted by I.A. DOBREITSER, in 1927 by towns of the European part of the USSR, the mortality rate per 100,000 inhabitants was 2.8 among men, and 1.5 among women with an average index of 2.1.

As to the fatality rate, i.e., the deaths calculated per 100 patients, in case of TF there is considerable variation in the individual epidemics in different countries. Thus, during 1918-1928, among the patients hospitalized with TF the lethality index varied from 2.2 (Palestina---out of 497 patients 11 died), to 5.4 (USA---47 died out of 809), up to 22.8 (Chile---8511 died out of 37,236 patients), and 29 (Egypt---51,832 died out of 177,723).

According to the much more accurate long-range data of the Red Council Hospital in Moscow, in the different periods the TF lethality varied at the following rate: 1902-1910--from 10.8 to 18%; 1919-1922--from 7.5% to 10.5%; 1923-1928--from 5.1 to 9.6%.

During the Second World War, the lethality of epidemics in foreign countries is reflected in the following indices: Iraq...9.8%; Iran...12%; Naples...13.7%; Cairo...14.4-21.1%. In the USSR among the hospitalized TF patients the lethality varied from 4.2% to 5.9% to 10.7% to 12.5%, with an average lethality of 6.1% in the Army (N.I. TAGOZA, 1955).

TABLE 2

TYPHUS FEVER IN NAPLES (ITALY) December 1943 - February 1944  
(Quoted after SNYDER, 1953)

Age in Years	Number of cases	Lethality, rate %
Less than 3	38	2.6
3-11	224	1.3
12-20	387	4.9
21-29	213	9.8
30-38	238	13.4
39-47	175	32.5
48-56	99	36.3
57-65	37	59.4
66-74	10	50.0
75	2	100.0
<b>TOTAL</b>	<b>1,423</b>	<b>13.9</b>

The lethality rate and age characteristic for TF are given in Tables 2 and 3.

As it can be seen from Table 2, the lethality rate of TF is the lowest in childhood. Further on it gradually grows, reaching its peak values in elderly subjects and especially in old people.

TABLE 3

TYPHUS FEVER IN CAIRO (EGYPT)  
January 1943 to August 1944 (Quoted after SNYDER, 1953)

Age in years	Men		Women	
	Number	Lethality Rate	Number	Lethality Rate
16-20	1,247	9.6	689	8.7
21-25	1,363	15.2	586	10.4
26-30	988	25.5	540	15.7
31-35	598	30.8	377	18.3
36-40	422	33.6	232	29.1
41-48	264	47.0	135	32.6
<b>TOTAL</b>	<b>4,882</b>	<b>21.1</b>	<b>2,559</b>	<b>14.4</b>

While illustrating similar ratios of TF lethality and age, Table 3 also shows that in men the mortality rate of this infection (21.2%) is considerably higher than in women (14.4%).

With the data quoted for Cairo in regard to the relationship of TF mortality rate to sex, the observations of the former SOKOL'NICHESKY Hospital in Moscow are in full agreement. In this hospital, during 1905-1908 the TF lethality in men varied within 13.5% and 20%, while the lethality in women was

from 12.3 to 13.5%. Thus, the reduced TF mortality in women compared to men (see above) depends not only upon their lower morbidity rate, but also upon a lower mortality rate, i.e., obviously upon a greater resistance of the female organism to TF infection.

As it was already shown in correspondence with the data of Table 2, the minimum TF lethality rate is observed in children. This situation should be however more precisely stated. If, as this was observed in the 1919-1922 USSR pandemic, TF afflicts children in their early childhood, then, especially in breast-fed infants, the mortality rate can be very high. Thus, according to the data of I. YA. VINOKUROV (1921), in Odessa during 1919-1920 the TF lethality rate among infants reached 21.7%, in children of the 1-4 year age group it was 4%, and only in the 5-9 year age group did it drop to 1.5%.

For epidemic TF, a definite seasonality is characteristic. The maximum figure of sickness cases is recorded in January to May, including a peak in April, and the minimum number of patients was noted in August-September. Table 4 presents the indices of monthly distribution of sickness cases in the USSR during the 1919-1923 epidemic period (averages--I.A. DOBREITSER).

TABLE 4

TYPHUS FEVER AND SEASONS OF THE YEAR											
MONTH	I	II	III	IV	V	VI	VII	VIII	IX	X	XI XII
Morbidity											
Indices	148	203	20	188	140	88	47	24	21	24	61 66

As it was already shown, in difference from the majority of other rickettsias, TF does not have an outside reservoir in Nature. Therefore, the term "endemic" in reference to TF can be used only conditionally in regard to areas where epidemic TF is constantly observed, owing to the sanitary conditions of life which to some extent provide a continued circulation of the virus in the louse-man-louse chain.

In the indicated sense, "endemic" distribution of TF after the Second World War was maintained in the extra-European areas to which belong: Morocco, Algeria, Tunis, Egypt, Abessinia, South Africa, Iran, Mexico, Guatemala, Columbia, Bolivia, and Chile.

In the USSR, just as in the majority of other East-European states, including also Poland and Rumania which were very strongly afflicted in the past, in recent years TF is recorded in the form of sporadic sickness cases which correspond to Brill's disease (See below) in the nomenclature of the International Nomenclature of Diseases (VOZ).

BASIC INFORMATION ON THE PATHOGENIC AGENT OF TYPHUS FEVER

The generally accepted pathogenic agent of TF, as we have already mentioned, is the *Rickettsia prowazeki* (RPr) (DA ROCHA LIMA, 1916) which together with the agent of rat TF or Mooser's rickettsia (*R. mooseri*, MONTEIRO, 1931), forms the subgenus *Rickettsia in sensu proprio* in the overall taxonomic system of rickettsias (Subgenus: *Rickettsia*, PHILIP, 1953). This corresponds to the group of TF rickettsias of the previous nomenclature.

Similarly to other rickettsias, the *R. prowazeki* are characterized as intracellular bacterioid, immobile Gram-negative microorganisms of very marked pleomorphism.

According to the summary data of P. F. ZDRODOVSKII (1948), which also include his original observations, in correspondence with their microstructure, which is differentiated by special staining, in RPr the following morphological types or variants are distinguished (Figure 1):

1. Type a, or coccoid rickettsiae, in the shape of very fine ovoids or ellipsoids, of a diameter less than, or about 0.5 micron, frequently forming diplococcic forms in "dumb-bell" shape, and occasional short chains, sometimes intracellular conglomerates in the shape of microcolonies. Cytomorphologically, they are homogenous, monococcal formations. These forms, while they determine the main morphology of RPr, are especially characteristic for intensive multiplication in the cytoplasm of affected cells (Figure 1 and 2).

2. Type b, or the rodshaped rickettsiae, in the form of short rods, of about 1-1.5 micron in diameter. Cytomorphologically they are bi-coccal (bi-granular) formations with polarly arranged granules which are connected with pallidly stained protoplasma. The rodshaped forms, just as the coccoid ones, are most frequently met with in RPr, and as the preceding forms, they represent an intensive multiplication of the pathogenic agent in the cells (See Figure 1 and 3).

3. Type c, or bacillary rickettsiae, in the shape of oblong, usually curved bacilli of about 3-4 microns in diameter. Cytomorphologically they are defined either as bi-granular formations with polarly arranged granuli, or even as quadri-(or sometimes tri-) granular rods, with two polarly arranged granuli. They correspond to rather sluggish multiplication, occupying an intermediate position between the preceding a and b forms, and the below described d forms.

4. Type d, or threadlike rickettsiae, in the shape of long, fantastically curved threads, or coarse spirilla in the size of 20-30-40 microns, and larger, which not infrequently form peculiar knots in the cellular plasma. Cytomorphologically they are multi-granular formations which represent sort of mycelia made of the a and b forms, or with the presence of evenly distributed granular a forms in their protoplasma. They are characteristic for initial phases of infection and for delayed types of reproduction (See Figure 5).

Essentially, the variegated morphology of RPr is evidently an expression of the different tempo of their growth and multiplication, while as P.F. ZDRODOVSKII showed in a case of pulmonary rickettsiasis in mice (1948) and as it was later corroborated by M. YU. MOROZOVA (1962) in cell explanations, a subsequent change of forms from a to d can occur with an intermediate lysis of the a-population which by all findings corresponds to the formation of ultra-filtrable forms of RPr.

RPr is multiplied by transverse division. P.F. ZDRODOVSKII (1948) distinguishes here an ordinary multiplication by division of the small forms with the formation of homogeneous populations, and a "mycellar" multiplication by fission of the thread-like forms.

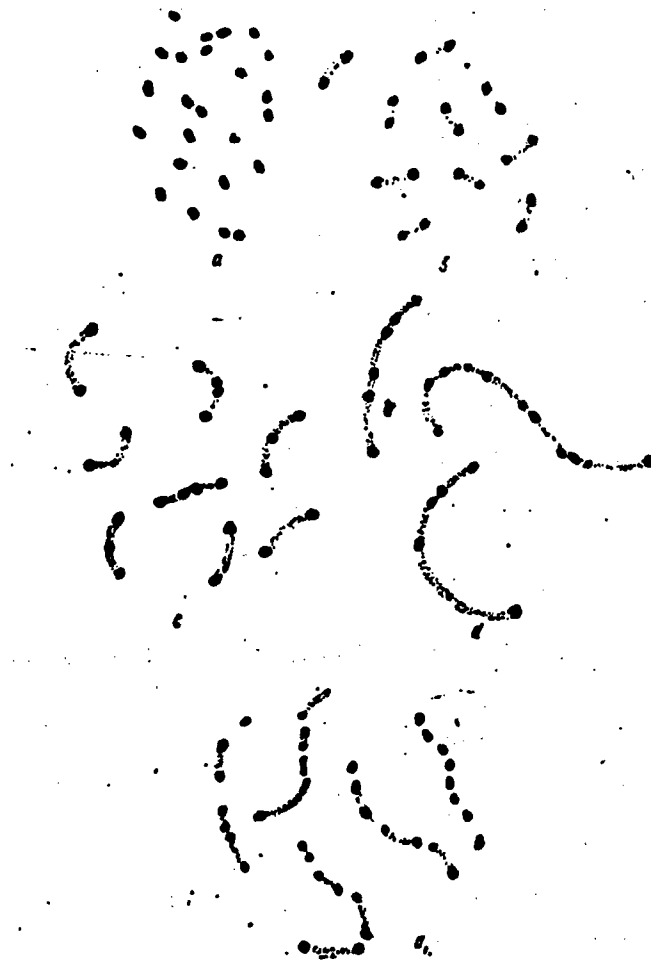
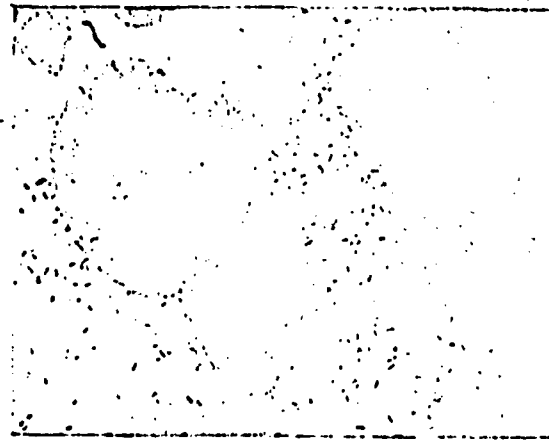


FIGURE 1: Sketches of *R. prowazeki* preparations according to BOUIN, and stained after ROMANOWSKY.

- a. coccoid forms; b. bacilloid forms; c. bacillary forms; d. threadlike forms;
- e. division of threadlike forms.



FIGURE 2: Microphotogram. Smear from the intestine of infected lice. Coccoid rickettsiae.



**FIGURE 3:** Microphotogram. Peritoneal rickettsiasis in guinea pigs. Smear from the tunica vaginalis of the testicle. Cells of the mesothelium filled with rickettsiae. Rod-shaped rickettsiae.



**FIGURE 4:** Microphotogram. Peritoneal rickettsiasis in guinea pigs (fatal case). Smear from the peritoneum. Bacillary rickettsiae.



The microstructure of RPr, just as that of the other species of microorganisms of this category, is similar to the structure of bacteria. In particular, microchemically a membrane of lipid capsule and protoplast is differentiated in them which contains granules of chromatinoid nature (A.V. RUMYANTSEV and E.P. SAVITSKAYA, 1948). Meanwhile, with the aid of basic staining, under ribonuclease control, the presence of ribonucleic acid is shown (RIS and FOX, 1949). With the removal of the membrane in RPr, a "soluble" antigen is released (SHEPARD, WYCKOFF, 1946).

For the chemical composition of RPr a high content of nucleic acid is characteristic together with a high content of lipoids and a small content of carbohydrates (V.I. TOVARNITSKII and collaborators, 1946).

The main physiological characteristic of RPr, just as also of other pathogenic rickettsiae, is generally related to their intracellular parasitism, with their inability to multiply in acellular media and on dead tissue substrates. As a difference from the rickettsiae of the tick-borne group (subgenus *Dermacentroxenus*, WOLBACH 1919), the RPr, colonize only the cytoplasm of the affected cells and are never multiplied in their nuclei. RPr. find the optimum of their vital activity in presence of lowered metabolism of the colonized cells, which is illustratively shown in appropriate experiments. Thus, they multiply well, for instance, in chick embryos (and in the lungs of rodents) at a temperature reduced to 35° - 36° and lower, but their growth is inhibited when the temperature is increased to 40°. However, even at 40°, rickettsiae can well develop if into the incubated egg a small amount of potassium cyanide is introduced (at a concentration of  $8 \times 10^{-5}$  -  $10^{-4}$ , which reduces the oxygen need. Similarly, the growth of the agent in chick embryos is activated by small amounts of sodium fluoride which inhibits tissue metabolism. The same effect is produced by lesion of embryos submitted to x-ray irradiations (D. GREIFF and H. PINKERTON et al, 1944, 1945, 1957).

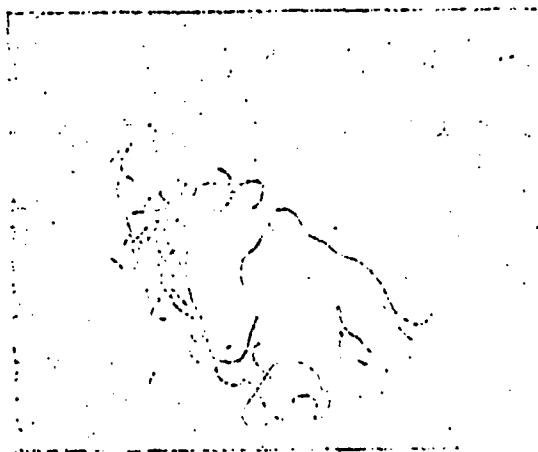


FIGURE 5: Microphotogram. Peritoneal rickettsiasis in guinea pigs. Smear from the peritoneum. Thread-shaped rickettsiae.

As to the type of respiration, RPr (but again in analogy with other species of rickettsiae) are aerobic. However, their respiration has a peculiar character: ---they actively oxidize glutaminic acid and behave indifferently toward glucose (M. BOVARNICK, 1949; BOVARNICK and SNYDER 1950). By oxidizing the glutamates, the rickettsiae convert adenosine-diphosphate (ADP) into energy-rich adenosine-triphosphate (ATP) which is also a power reserve (BOVARNICK, 1956).

RPr form specific toxic substances which are however closely tied to the body of live rickettsiae, and, in difference from bacterial toxins, they are not excreted by the rickettsiae into the external environment. At the same time in the body these toxic substances, as true toxins, provoke the formation of specific neutralizing antibodies analogous in all respects to antitoxins. The toxic substances are very labile. They are inactivated at 60°, by formalin; with ordinary storage, however, they maintain their antigenic properties, i.e., as if they were converted into a specific antitoxin. Relatively well are preserved the toxic properties of rickettsiae only at low temperatures (-70°). At investigating the toxic properties in case of need in laboratories, a suspension of live RPr. of any derivation is used (louse or lung virus, egg culture---see below). The investigation of the toxic properties itself is done on mice which are killed 24 hours after intraperitoneal or, still better, after intravenous administration of a native suspension of live rickettsiae of sufficient concentration. The first knowledge on the toxic properties of rickettsiae was acquired with egg cultures of Mooser's rickettsiae, i.e., of the agent of rat TF (GILDEMEISTER and HAAGEN, 1940), and a year later they were also demonstrated for RPr (R. OTTO and R. BICKHARDT, 1941).

Together with the toxic properties of RPr, hemolytic properties were also discovered which, by analogy with the first properties, are tied to live microorganisms, and at immunization they cause the formation of corresponding neutralizing antigens in the organism (D. CLARKE and J. FOX, 1948).

As it was repeatedly said, by analogy with the viruses which are obligate intracellular parasites, RPr do not reproduce in ordinary acellular media used for the cultivation of bacteria, but they are abundantly accumulated under laboratory conditions in the stomach of contaminated lice (method of DA ROCHA-LIMA and WEIGL, 1916, 1919), in the lungs of intranasally infected rodents, especially mice (method of CASTANEDA, DURAND and SPARROW, 1940), and in the vitelline sacs of developing chick embryos (method of COX, 1940). RPr can be also cultivated in different surviving tissues (for instance, in the tissues of chick embryo), which are suspended in liquid Tirole-serum medium (method of H. MAITLAND and M. MAITLAND, 1928), or placed on the surface of Tirole-serum agar (method of ZINSSER and collaborators, 1939). In recent times, by analogy with other species of this group of microorganisms, RPr was successfully raised in cultures of various cells parietally growing in appropriate liquid media (M. YU. MOROZOVA, 1963).

For mass production of RPr under laboratory conditions, at present they mostly use either their accumulation in the lungs of rodents, especially in mice (lung cultures), or even their breeding in vitelline sacs, more accurately in the cells of the vitelline sac of developing chick embryos (egg cultures). Both indicated methods assure the possibility of accumulating RPr. in unlimited quantities.

The egg cultures, although somewhat more complicated for obtaining, have however the advantage that they do not contain bacterial admixtures which are practically unavoidable in the pulmonary culture of rickettsiae, since the latter accumulate in the open system of the lungs.

Just as also the bacteria, the pathogenic rickettsiae, first of all the RPr., display the phenomenon of variability, which is shown most illustratively in regard to their virulence. Thus, in 1941 in Madrid from a patient with a severe case of TF a strain of RPr was isolated which on the 11th passage in chick embryos chipped off the variant E which has a lower virulence for laboratory animals and man (GLAVERO DEL CAMPO, and F. PEREZ GALLARDO, 1943). Further on, this variant was stabilized as the low-virulent E strain of RPr which at present is thoroughly studied and approved on people of the USSR and abroad as a live vaccine against TF (E.M. GOLINEVICH and collaborators, 1957 - 1962; FOX, J. MONTOYA et al., 1959).

In 1957, A.V. PSHENICHNOV and O.N. SHEVELEVA published their findings on the loss of virulence for guinea pigs in two fifth of the RPr strain under the effect of its continuous passages in lice for 11½ years.

Finally, in recent times, the prospects of an experimental transformation of RPr were outlined. Thus, in his laboratory, H. PRICE (1958) describes the successful transformation of RPr. into R. mooseri under the effect of DMA, contained in the body of the latter.

Serologically, by their antigenic structure, RPr. are distinguished from rickettsiae of the tick-borne spotted-fever group (Type D. sibericus), the rickettsiae of Q-fever (R. burneti) and the rickettsiae of tsutsugamushi (R. orientalis), but they are similar to the rickettsiae of rat TF (R. mooseri) due to a thermostable anti. a common with the latter (CASTANEDA, ZIA, 1933). However, the presence of a type-specific antigen in RPr. makes possible to serologically differentiate them from R. mooseri by the preponderance of the homologous titre of immune serum over the heterologous titre. An illustration of the serological differentiation of RPr from other rickettsial species is shown in Table 5. (after the data of E.M. GOLINEVICH).

TABLE 5

Immune sera	Antigen	Serum dilution in the complement binding reaction (BR)							
		1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280
Prowazek	R. prowazeki	4	4	4	4	4	4	4	3
	R. mooseri	4	4	4	3	2	-	-	-
	D. sibericus	-	-	-	-	-	-	-	-
	R. burneti	-	-	-	-	-	-	-	-

RPr are unstable under the effect of heat and are killed in a liquid medium already at warming to 50°. On the contrary, they display a good hardness toward low temperatures, especially by preserving their vital capacity long at -60° - -70°. The survival capacity of RPr in liquid media under ordinary thermal conditions is very limited. It is somewhat better in protein media (for instance, in skim milk), but even here at a temperature of 23° - -26° it does not last more than 48 hours.

In an acid medium (pH 4.0 - 6.0) the rickettsiae are killed very quickly. In difference from liquid media, in dry substrates, RPr display a rather good resistance in external environment, but this chiefly refers to rickettsiae excreted with the fecalia of infected lice (see below). Especially well do the RPr preserve their vital capacity in lyophilic exsiccation in protein media with subsequent storage in vacuum in a refrigerator at 0 to 5°. Thus, according to the data of E.M. GOLINEVICH, under these conditions, rickettsiae are preserved not less than eight years. It is important to mention that at subsequent moistening of the dry substrates, RPr are quickly killed.

RPr, like the unstable vegetative forms of bacteria, are made easily harmless with disinfecting substances (Chloramino, formalin, phenol, lysol, and alkalies, and so on) at customarily used concentrations.

### TYPHUS FEVER INFECTION IN LICE

The question of TF as a sickness of people are epidemiologically inseparable from this type of rickettsiasis in lice which are the only source of the TF infection of people. Therefore, in the present review we give a rather comprehensive information on lice and the infectious process at their contamination with RPr.

#### BRIEF INFORMATION ON LICE AND THEIR BIOLOGY.

(FOOTNOTE: In the description of lice, the articles of E.N. PAVLOVSKY (1928 - 1958) were mainly used).

In the definition of E.N. PAVLOVSKY (1958), lice are insects of the order of Pseudorhynchota which are parasitic exclusively on mammals whose blood they drink. Moreover, all species of lice are monoxenous, i.e., each species of lice is parasitic only on a particular species of animal. On man, three species of lice of the Pediculidae family are parasitic: -- *Pediculus vestimenti* = the body louse; *P. capitis* = the head louse, the *Phthirus pubis* = the crab louse. The main carrier of TF is the body louse, and probably also the head louse. Therefore, further on we give the characteristics of these two species of lice. Both species are hematophagous, and very closely related to each other. It is enough to say that a crossed interbreeding is possible between them, while according to the data of V.V. ALPATOV (1945), the head louse can change into body louse under certain conditions. Therefore, some authors consider both louse species as subspecies of one species, *Pediculus humanus* (V.V. ALPATOV et al., 1945; E. MARTINI, 1940).

The head louse is of gray color, with darkly pigmented spots on the sides of its chest and abdomen. The size of the male is 2-3 mm, of the female 2.4 - 4 mm. Ordinarily it lives on the scalp.

The body louse (= clothes louse) is of light gray color. It is longer than the head louse. The male is 2.1 - 3.72 mm, the female 2.2 - 4.75 mm. It lives in the folds of linen and clothes, passing over to the body for blood-sucking only.

Lice are bisexual. The mating of body louse is really for 15-20 days, and of the head louse for 7-12 days. The fertilized female lays eggs (=nits) (Figure 6), attaching them with an excreted fluid to the fibres of a fabric (body louse) or to hairs (head louse). The rather important conditions of egg laying are sufficient satiation of the females, and temperatures within 20° to 37°, with an optimum at 32°.

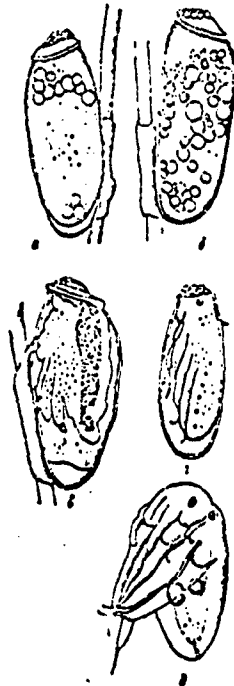


Figure 6. - Ripening of the embryo in louse nits, according to NUTALL (after E.N. PAVLOVSKY, 1948).

a, b, c, d, e, ... subsequent stages of embryonal development.

The body louse lays 6-14 nits daily, and during its entire life 200-300 nits. But the head louse deposits not more than 4 nits, and during its whole life not more than 141 nits.

The length of embryonal development of lice in the nits depends upon the outside temperature. The optimum for development is  $30^{\circ}$  -  $31^{\circ}$ . If the temperature is  $40^{\circ}$  -  $45^{\circ}$ , or lower than  $22^{\circ}$ , the larvae do not hatch. The minimum time of development at  $36^{\circ}$  -  $37^{\circ}$  temperature is 4 days, at  $35^{\circ}$  6 - 8 days, at  $30^{\circ}$  7 - 14 days, and at  $25^{\circ}$  16 days. In case of alternating cooling and heating (for instance, with periodic putting on and taking off the clothes) the development of lice in the nits is delayed to 5-6 weeks. From the nits, larvae will emerge (Figure 7) which pass through three stages of development, by coming to an end as sexually mature forms (Figure 8). The length of the postembryonal development depends upon many conditions (temperature, moisture, feeding, individual peculiarities) and it is equal to 12 days as an average.

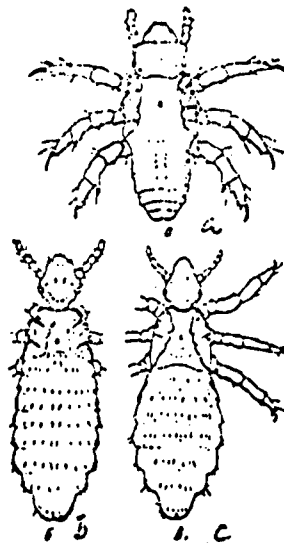


Figure 7. - Transformation of lice (after E.N. PAVLOVSKY, 1958).

a...larva I; b...larva II(nymph); c...larva III(nymph II).

The sexual cycle of the development of lice is presented in the following form: (1) embryonal development...4 days to 6 weeks; (2) larva of Stage I...3-5 days; (3) larva of Stage II...4-5 days; (4) larva of Stage III...3-4 days; (5) sexually mature form. The life duration of a female body louse is 46 days, of a male 32 days. The same for a female head louse is up to 38 days, for a male head louse 27 days. The life cycle of development of a body louse, from the moment of egg laying to the laying of eggs by the female which developed from this egg at its habitation on man, equals 16 days. To the end of its life, the female body louse can leave a posterity up to 4100 individual specimens.

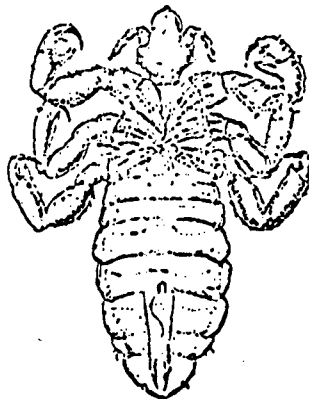


Figure 8. - Body louse. Male (after E.N. PAVLOVSKY, 1948).

As already mentioned above, at all stages of postembryonal life, the body and head louse feed exclusively on blood. Here, according to the data of G.M. DUTOVA (1965), the body louse sucks up to 1.4 mg of blood at once, and the head louse 0.5 - 0.65 mg. The body louse sucks blood 2-3 times a day, while under laboratory conditions the head louse needs four feedings (according to the observations of G.M. DUTOVA). The body louse can tolerate hunger up to 10 days at 10° - 20° temperatures, and it quickly dies at higher temperature, for instance in 12 hours at 40°, and in 1-2 days at 37°.

Lice are very sensitive to high temperatures, and in dry air at 54°, they die in 35 minutes.

According to G.S. MOSING (1937), the microclimates of the places of habitation of body lice, depending upon the seasonal clothing, are warm in winter, while in the summer they are cool, since with warm winter clothes, the temperature is about 30° between the clothes and the body surface, i.e., it corresponds to the optimum temperature, while with light summer clothing the temperature is considerably lower. Hence, there is a massive multiplication of lice in winter, and a relatively scanty one in summer.

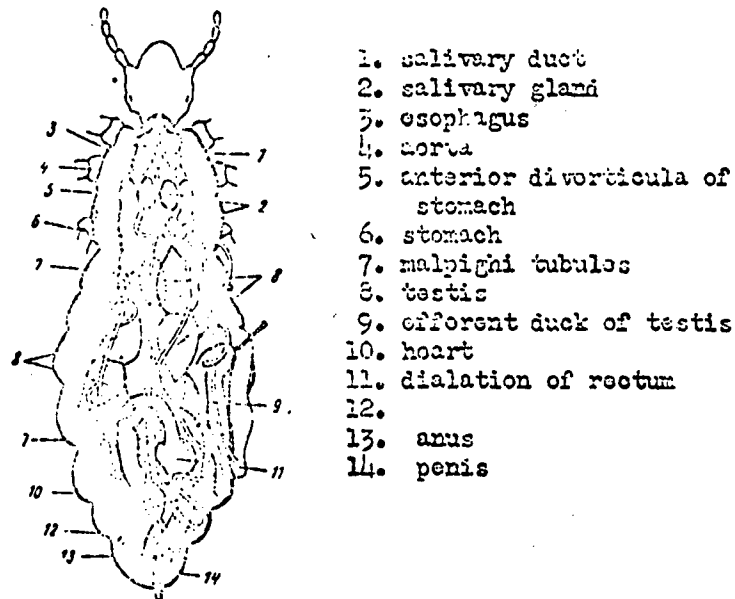


Figure 9. - Anatomy of the internal organs of body louse (Male) - (After BRUNET, 1949).

#### LABORATORY CULTURES OF LICE.

With the provision of a regime corresponding to their biology, body lice multiply very well under laboratory conditions. The here pertinent technical procedure, elaborated by A.V. PSHENICHENOV and B.I. RAIKHNER (1945) assure the laboratory cultivation of body lice in unlimited quantity and for any length of time.

The basic conditions for a successful cultivation of body lice are the following factors:

(1) A production flock is formed for healthy lice at the ratio of 1:1 of males and females.

(2) The lice are kept in crystallization cups or other cups (dishes) at a density of 100 larvae per 4-5 mm<sup>2</sup> and 100 adult specimens per 3.5 - 4 cm<sup>2</sup>.

(3) Egg laying (nit laying) is produced on human hairs placed in the cup, and the hatching of eggs is provided in a thermostat at 35° - 37° at 80%-85% humidity. Under these conditions, larvae hatch from the nits in 5-6 days.

(4) The larvae and the adult forms are kept in cups at the indicated density of specimens in a thermostat at 32°-33° and 80%-85% humidity. In case of double feeding under these conditions, the first molting occurs after 4-5 days, the second after 10-11 days, and the third after 13-14 days.

(5) Feeding of the lice maintained under the above indicated conditions is done on donors twice daily, morning and evening. Special small "boats" are used for feeding.

The life duration of laboratory male lice is 20-25 days. According to the findings of our laboratory, louse breeding according to A.V. PSHEMNICHENOV's method is entirely successful. Here, fully satisfactory results are also obtained with their single feeding on donors. In recent years, however, in our laboratory body lice are used which were adapted to feeding on the blood of rabbits (this race of lice was detected in the laboratory of A.V. PSHEMNICHENOV). This louse race, having been adapted to feeding on rabbit's blood (just as also a similar race obtained by FULLER), considerably simplified the work with body louse in conformity with the tasks of IF research.

Recently, in agreement with the findings of A.V. PSHEMNICHENOV and A.V. GREMBOVSKAYA (1960) in our laboratory head lice are also successfully cultivated at 32° - 33° degree of temperature with humidity within 47%-50%. In difference from body lice, head lice require, however, four daily feedings on donors. The hatching of the larvae from eggs (nits) is seen here within a range of 72% to 85% on the 6th to 7th day after egg laying, and the length of the whole developmental cycle is 18-20 days. The percentage survival of head lice in laboratory cultures is noted within the range of 35-40 to 50 days (G.M. DUTOVA, 1963).

#### TYPHUS FEVER INFECTION IN LICE

Below we expose the more important experimental data on IF infection in body lice, including more recent observations on the same infection in head lice.

The body louse is easily infected with RPr at all stages of its post-embryonal development, i.e., at the larval stages and adult stages. Under natural conditions, lice are infected through blood sucking on IF patients. Under laboratory conditions, lice can be infected by three methods:

1. by feeding on sick persons;
2. by inoculation of infectious material by amicrocannula (through thin capillaries) according to WEIGL, and finally,
3. by feeding infected blood through a membrane with using an epidermo-membrane taken from a human cadaver (after PSHEMNICHENOV), or skin taken from a one-day old chick (after FULLER, MURRAY and SNYDER<sup>1</sup>).

1) Recently one of the USA firms released artificial membranes for infective feeding of lice.



More recent works on more precise study of the TF infection in body lice, which were carried out during 1942-1948 in the laboratory of A.V. PSHEMENICHNOV, were accomplished with the employment of infected feed offered to lice through an epidermal membrane. The essence of this method consists in the following:

In a special feeder in form of a small round metal cup of 3-4 cm diameter, we pour defibrinated or citrated blood of a TF patient, or blood of a normal person with admixture of TF virus of any derivation at an appropriate concentration. The ring-shaped lid of this feeder is provided with a stretched out epidermal membrane which comes in contact with the free surface of infected blood. Head lice subjected to infection, or, still better, their larvae are put upon this membrane through which they readily suck themselves full with infected blood. One session of infective feeding lasts about 20 minutes as an average (with possible variations up to 1-2 hours), assuring a simultaneous infection of the majority of lice (better in the larval stage).

In our laboratory the mentioned method of A.V. PSHEMENICHNOV was used for a number of years with continued success (P.L. SOLITERMAN), but lately, without prejudice to the results, the epidermal membrane, which was taken by a special procedure from the skin of human cadavers, was substituted with the skin of a one-day old chick. Through the latter and most accessible membrane, as experience proved, 70-80% of the body lice become infected.

Let us mention briefly only the more important results of TF infections which were obtained with infection of body lice according to the method of A.V. PSHEMENICHNOV in the laboratory under his guidance.

These researches exclusively established first of all the high grade susceptibility of body lice to TF infection. Thus, a louse-infecting dose (abbreviated: DIL) for larvae which get satiated by sucking about 0.05 mg of blood, equals 1/3,000,000 part of the intestinal content of Nymph II (i.e., larva of Stage III) at the height of infection, which, according to the data of A.V. PSHEMENICHNOV is approximately equal to 10-20 rickettsiae.

As it was established in his time by G. ROCHA-LIMA (1916), in lice the multiplication of rickettsiae is limited to the stomach. Here, for RPr a colonization and multiplication in the epithelium is typical, which as a result is destroyed with release of the agent into the intestinal lumen and with its subsequent excretion into the external environment with the excrements (fecal TF virus). The intensity and speed of development of TF rickettsiasis depends upon the size of the infective dose and the temperature of the outside medium. The importance of the latter, according to the findings of A.V. PSHEMENICHNOV's laboratory, is illustrated by the following relations (Table 6).

Table 6

Suscepti- bility to Infection	Temperature				
	14-16°	26-28°	31.5-32°	33-34°	36-37°
1. Time of detecting the rickettsiae (days)	17-31	11-25	4-6	3-4	3-4
2. Percentage of infection of lice	6-13	20-60	100	100	100
3. Intensity of infection	+++	++++	++++	+++	+++

In corroboration of, and as a supplement to the above, in a work published in 1959, A. HERZIG-WEIGL and S. KRYMSKI showed that at 22° on the 9th day, rectal infection of lice causes only a weak infection (Figure 10), which afterwards will gradually become stronger, reaching its maximum on the 12th day, when 100% of the gastric epithelial cells gets attached (Figure 11). According to the data of the same authors the body louse is infected,

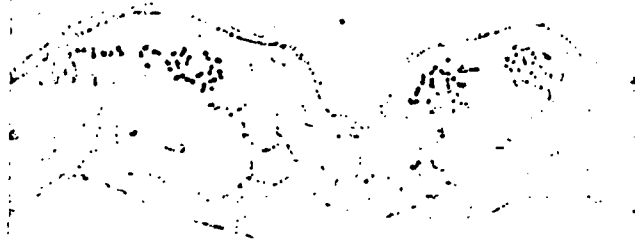


Figure 10. - *Rickettsia prowazekii* in the gastric epithelium of the louse at the initial stage (24 h) of infection (after R. WEIGL).



Figure 11. - Final stage of infection of the louse stomach by *Rickettsia prowazekii* with massive destruction and disquamation of the epithelium (after R. WEIGL).

even at 16° with lesion of the epithelium of the stomach on the 6th day in 20%, on the 9th day in 40%, and on the 15th day in 100%. Moreover, the authors permit the possibility of development even at lower temperatures, which---as we have already seen---was also proved in the experiments of A.V. PSEENICHNOV (susceptibility of lice to infection at 14° - 16°).

In contaminated lice which are kept at a relatively low temperature, a latent infection develops. In case of their transportation to more favorable thermal conditions, the infection is activated, and is accompanied by abundant multiplication of RPr (S.N. RUCHKOVSKI, 1934; A.V. PSEENICHNOV, 1943).

The TF infection is lethal for the infected body lice. Depending upon the intensity of infection, the life duration of the infected lice can widely vary, within 3 and 31 days (A.V. PSEENICHNOV). In case of an infection of

moderate intensity, the lice get sick on the 12th-13th day after contamination, and they die on the 15th to 18th day (LEPINE, 1938). The cause of death of infected lice is evidently the rickettsial intoxication and the destruction of the epithelial lining of the stomach. The passage of lice through sickness is accompanied by a red imbibition of their bodies with hemoglobin.

In case of rather strong infection of lice on the epidermal membrane, with their subsequent maintenance at  $32^{\circ}$ - $32.5^{\circ}$ , and with a relative humidity of 70-80%, the movement of rickettsial infection takes the following form in them.

In the first four days after inoculation, the development of rickettsiae in the lice goes on very slowly, and in this period the number of agents does not exceed 20,000 - 40,000 DIL. Further on, the infection progressively increases, and reaches its maximum on the 8th - 9th day, provoking a mass death among the insects. At this time, the number of agents reaches 2-3-5 million DIL in the larvae of Stage III, and up to 4 million in adult lice which---according to the findings of A.V. PSHENICHNOV, corresponds to approximately 30-50 million rickettsiae.

According to observations in our laboratory (P.L. SOLITERMAN), the dynamic change of morphology of RPr in the infected lice proceeds in the following form.

Appearing in the stomach of lice, at the end of the second or at the start of the third day after inoculation on the membrane, in this early phase of infection the rickettsiae have the form of bipolar rods b and rather long bacilli c. Five to six days after infection, and sometimes even earlier, the rickettsiae give the image of sharply marked pleomorphism. Beginning from the 7th-8th day, the indicated diverse forms are exchanged for a homogeneous population of fine bacilloid rickettsiae b, which still later (9th to 10th day) are replaced by coccoid forms a and by still finer "dust-like" formations, obviously being the products of a partial lysis (P.L. SOLITERMAN, 1948).

Such is the general outline of the picture of morphological changes of RPr which are noticed in infected body lice during infection, changes similar to those which can be observed in the lungs of infected mice (P.F. ZDRODOVSKII, 1943) and in wall-standing cell cultures (M. YU. MOROZOVA, 1953).

The head louse, as this had been shown at its time by J. GOLDBERGER and J. ANDERSON (1912), and by Da ROCHA-LIMA (1916), and lately corroborated by Soviet authors (A.V. PSHENICHNOV, and A.V. GREKOVSAYA, 1962; G.M. DUTOVA, 1963), in analogy with the body louse, was successfully infected with RPr in experiments.

In our laboratory, G.M. DUTOVA showed that rickettsial infection in the head louse can be made in approximately 50%-60% both by their inoculation through a micro-onoma according to WEIGL's method, and with feeding them on a membrane from a young chick's skin. At  $32^{\circ}$ - $33^{\circ}$  and a humidity of about 50%, the infection of the stomach in the head louse is discovered from the 4th-5th day, reaching its maximum on the 9th-11th day. In experiments with intranasal infection of mice, and intraperitoneal and conjunctival infection of guinea pigs, the TF virus displayed fully virulent properties. As in body lice also, in the infected head lice the RPr is excreted with the fecalia at a virulence of the latter for guinea pigs in a dose of  $10^{-5}$  at intraperitoneal infection.

Thus, according to experimental findings, under suitable conditions the head louse can be obviously a source of TF infection for man. For the understanding of the mechanism of transmission of the TF virus from lice to man, it is decisive that in infected lice, the rickettsial virus is absent from the salivary glands (Da ROCHA-LIMA, 1916), and is excreted from the intestines with

the excrements. Therefore, infection of a human being cannot occur by a louse bite as such, and the actual source of TF infection for man (not to consider the crushing of lice) is the fecal virus excreted by infected lice which penetrates into the organism through the injured skin (scratches) and through the mucous membranes (conjunctival sac of the eye, mucosal membrane of the respiratory pathways). Moreover, at the evaluation of the fecal rickettsial virus of infected lice as the main infective material for man, it is also necessary to consider that in a desiccated condition this fecal virus displays marked resistance. All the mentioned material requires that the question of fecal TF virus should be specially discussed.

#### FECAL TF VIRUS OF LICE.

Contrary to the original idea of Ch. NICHOLLE on the rapid death of the TF agent in the external medium (NICHOLLE, 1920), the researches of English authors showed already in 1922 that in the dry fecalia of lice the RPr preserves its infectiousness for 11 days (J. ARKWRIGHT and A. BACOT, 1922). These findings did not get proper attention, however, and their importance became evident only in recent years. It should be mentioned that in 1936 B. PALCINA (Warsaw) discovered virulent RPr in dried-out lice and their fecalia which were preserved in a refrigerator for 27 months---a period of time which caused a lot of skeptical attitude on the part of many authors (Cf. in LEPINE, 1938).

Later on, however, H. STARZYK (1936-1938), using biotests on guinea pigs, established that in the dried out fecalia of infected lice at 5° - 7° in ordinary atmosphere, RPr are keeping their vital capacity for 66 days.

The same author found in later investigations (1948) that the fecal TF virus on sheep wool with ordinary maintenance survives at keeping its virulence 12 months and 23 days.

The findings of Polish authors in regard to the resistance of RPr in the dry fecalia of infected lice, found later corroboration by many investigators who to a considerable degree made more precise a number of factors which have an effect upon the preservation of the fecal virus.

Thus, S. CHAO (1944) investigated the capacity of preservation of RPr in the fecalia of infected lice depending upon temperature and humidity. His obtained results are summarized in Table 7.

TABLE 7

Temperature	Humidity in %	Time of survival in days	Time of death in days	Remark
3-6°	60-70	20	41	Experiment at room temperature
10-20°	30-60	145	190	
25-28°	30-35	50	130	Humidity 75%
25-28°	75-90	19	25	

Thus, the quoted experiments of CHAO illustrate the effect of humidity upon the preservation of RPr in the fecalia of lice, with the most favorable influence of relatively dry air upon them at moderate temperature.

M. KITAKA and A. SHISHIDO (1950), in their turn, found that the fecal TF virus of lice shows an infectivity to guinea pigs two hundred and thirty-three days after keeping it at room temperature, but it proved to be inactive at its testing after a 121-day storage at 0° - 5°.

Finally, lately F. WEYER (1959) in the Hamburg Institute demonstrated that, on the preservability of RPr in louse fecalia, in addition to external conditions, the individual properties of Rickettsia strains may also have a substantial influence which strains can be stable or labile.

Thus, for the Cairo strain the author showed that at room temperature its fecal virus is maintained vitally capable and virulent for 35 days, with negative results of testing after 37-64 days. Here, in individual experiments, death of rickettsiae was detected also in shorter times (6-10th, 11-14th, 16-20th, 21st day). At the same time, in identical experiments with the Tunis strain, the storage of the fecal virus of lice was accompanied by the death of rickettsiae 21, 34 and 54 days later.

In a second series of experiments on the preservation of fecal virus of infected lice in a refrigerator, for the Cairo strain the rickettsiae were found in vital capacity until 75 days (14 favorable tests out of 16), while in similar experiments with the Tunis strain the rickettsiae were maintained in vital capacity only for 32-40 days.

Generalizing his personal observations, and the above quoted data of the literature, in his work WEIER comes to the following objectively founded conclusions:

1. RPr, excreted by infected lice into the outside environment with the excrements (fecal virus), under natural conditions displays very high resistance in comparison with any other of its forms, which in its turn is of great importance both for the preservability of the species of rickettsiae and for the infection of human beings with TF.

2. The vital capacity of RPr excreted with the excrement of infected lice depends upon individual peculiarities of the strain, temperature of the outside environment, and humidity. The optimum conditions for keeping fecal TF virus of infected lice are low temperature and dry air.

Probably, the surviving capacity of the fecal virus in external environment is also influenced by other, still unknown factors.

In supplementing the above, G.M. DUTOVA (1962-1963) showed in our laboratory that in the fecalia of infected head lice the RPr can be preserved in vital capacity in virulent condition for 3 months at room temperature 18° - 20°, in a thermostat at 33°, and in a refrigerator at 4° and at a variation of humidity within 47% and 60%. According to the data of L.N. ASTAKHOVA (1954), of the laboratory of A.V. PSHEMENICHENOV, in the fecalia of infected lice under similar conditions at room temperature the RPr can survive 125 days, and in a refrigerator it can survive for a whole year <sup>1)</sup>.

1) L.N. ASTAKHOVA: Materials on the Preserving Capacity of TF Rickettsiae. Candidate dissertation. Perm'. 1954).

### EXPERIMENTAL FORMS OF TYPHUS FEVER INFECTION IN ANIMALS

In the works on TF of research and applied importance, the experimental forms of reproducing this infection in animals occupy a prominent place. Hence, the related information in regard to various animals is briefly given in the following manner.

Rhesus monkeys - show a rather strong susceptibility to RPr., by becoming regularly infected not only parenterally, but also at putting the TF virus on mucous membranes, especially into the conjunctival sac of the eye. However, the susceptibility of monkeys to TF should not be overrated. Thus, e.g., at the immunization of monkeys with the attenuated (vaccinal) E strain of RPr under comparable conditions a worse effect is found than in guinea pigs. This is explained by the relative susceptibility of the Rhesus monkey to TF.

In case of an effective infection, after an incubation period of some length of time which varies according to virulence, dose and mode of introducing the agent, in monkeys a marked fever of many days' duration appears which usually remains the only symptom of the ailment, ending with full recovery with the presence of a well marked immunity to re-infection.

The TF infection in monkeys is accompanied by the formation of antibodies. These are manifested in a positive WEIL-FELI reaction with *Proteus* OX<sub>19</sub>, and in serological reactions with an antigen made of RPr.

Guinea pigs - display a proportionate sensitiveness to TF infection, more marked in comparison with Rhesus monkeys, but, according to the data of A.V. PSEVNICHNOV (1944), 50 to 100 times less so in comparison with man. In guinea pigs an experimental infection is produced by all methods of parenteral inoculation of TF material of any derivation (blood and organs of sick people and sick animals, intestine and its content taken from infected lice, cultures of RPr).

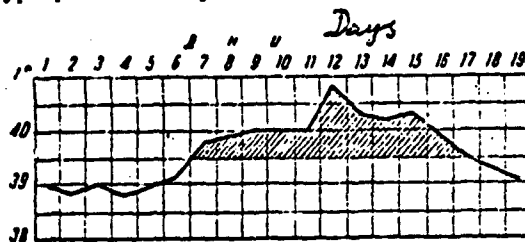


Figure 12. - Fever curve of the guinea pig. Experimental epidemic typhus fever. Passage infection with cerebral virus. Febrile reaction.

According to the findings of D. COMBIESCU (1941), infection also arises in guinea pigs when the TF virus is placed on the slightly injured skin (shaving, epilation), and when it is instilled into the conjunctival sac of the eye. Transplacental infection is also possible (COMBIESCU et al., 1933).

A generally accepted method of guinea pig inoculation is the intraperitoneal method.

In the inoculated guinea pigs the incubation period varies from 5-6 to 14-16 days and more, depending upon the peculiarities and adaptation of the virus, its dose, and the mode of inoculation. The febrile reaction of continuous or irregular type, with a rise in temperature up to 40° and higher (when the temperature is measured in the rectum with a veterinary thermometer) lasts 5-10 days as an average, with variations between 3-4 and 11-15 days (Figure 12). Sometimes in the inoculated guinea pigs asymptomatic forms of infection ("1' infection inapparente") are observed which run with normal temperature (CH. NICOLLE and S. LEBAILLY, 1919). Under experimental conditions, these inapparent forms can be reproduced by inoculating the guinea pigs with a mixture of immune serum and virus (DOERR, SCHNABEL et al, 1921).

According to the observations of A.V. PSHENICHENOV (1944), at the height of infection the TF virus is distributed in the following order: -- maximum concentration in the brain, suprarenals and spleen; considerably smaller concentration in the blood and internal organs. The virus is absent in the urine. The brain gets infected after 24-28 hours before the elevation of temperature. It keeps the infective capacity during the entire febrile period, and it can stay infective up to 20 days after the end of fever (quoted after LEPINE, 1938). According to the data of K.M. SINILAK (from the laboratory of G.S. MOSING, 1955), in inoculated guinea pigs, RPr can be found almost always in the kidneys by a biotest on lice up to the 21st day of convalescence, and in some cases they can keep on until the 3rd month (3 observations).

The brain of inoculated guinea pigs (in case of virulent TF virus) may contain up to 50,000 - 100,000 infective doses.

When male guinea pigs are inoculated with passage material (especially with brain), the scrotal phenomenon (rickettsial periorchitis) is usually absent, yet, in some cases in epidemic (or sporadic) TF, "intermediate" strains (*R. prowazeki* var. *intermedia*) are isolated from the patients, which at inoculation of guinea pigs will provoke the formation of a typical periorchitis with the accumulation of rickettsiae in the mesothelium of the tunica vaginalis of the testes and of the peritoneum, regardless of the character of infectious material inoculated into them (blood of patients, brain from passage animals) (E.M. GOLINEVICH, 1950).

TF virus strains which produce mild, transitory forms of periorchitis were also described by ZINSSER (1932).

Together with this, the intraabdominal introduction of sufficient doses of RPr suspension of any derivation in male guinea pigs, e.g., a suspension of rickettsiae bred in culture (quoted after ZINSSER, 1932), but even a suspension from the intestine of infected lice (M.K. KRONTOVSKAYA, and E.P. SAVITSKAYA, 1941), will regularly provoke a specific periorchitis with accumulation of rickettsiae.

The Weil-Felix reaction with *Proteus* OX<sub>19</sub> antigen is absent in TF guinea pigs, but their serum specifically agglutinates RPr. and gives a complement binding reaction (CBR) with an antigen prepared from these rickettsiae (particularly regularly 3-4 weeks after inoculation). We mention favorably that, under the control of complement binding reaction as an indicator of infection, it is customary to titrate on guinea pigs the minimum infective dosage of TF virus (e.g., the egg culture of RPr, which for this purpose is used in decreasing dilutions...10<sup>-3</sup>, 10<sup>-4</sup>, 10<sup>-7</sup>, 10<sup>-8</sup>).

The initial production of TF infection of guinea pigs is best done with their intraabdominal inoculation, using 3-5 ml blood taken from the initial period of fever (it is best during the first five days of sickness). Inoculation with an emulsion made from a clot of coagulated blood not only simplifies, but also increases the chances of producing an infection (GIROUD).

At the initial infection with blood from a patient, in the inoculated guinea pigs the fever of typhus is usually detected after a long incubation of 14-16 days, a period which is gradually shortened at subsequent brain passages; meanwhile it does not reach a fixed value.

The passing of virus from pig to pig can be done regularly with subsequent intraabdominal inoculations of a brain emulsion (1 ml of a 5-10% emulsion) taken from a guinea pig which was killed at the height of the febrile period. Stable strains of TF can be thus maintained on guinea pigs indefinitely long. Thus, e.g., the RABT strain, isolated from a patient in the laboratory of CH. NICOLLE in April 1924, was maintained continuously and without noticeable changes in passages on guinea pigs until the date of the author's jubilee in 1959, i.e., for the length of 35 years (SPARROW, 1959). The same is true for the generally known strains of OTTO, WEIGL and so on. Brain-passage virus from guinea pigs keeps its pathogenic properties also for man, as this was proved in 1921 by E. SPARROW in Warsaw in a test on self-infection with a guinea pig brain emulsion of the 35th passage.

In conclusion, it should be pointed out that the intraabdominal inoculation of guinea pigs with RPr in sufficient dosage, regardless of the origin of rickettsial material, in addition to the above described periorchitis (in males), will cause a specific peritonitis with multiplication of rickettsiae in the mesothelial cells of the peritoneum and the tunica vaginalis (P.F. ZDRODOVSKII, 1943).

In guinea pigs, peritoneal TF rickettsiasis runs with fever which is manifested on the 2nd-3rd day after inoculation, reaches its maximum on the 4th-5th day and then it ceases by the end of a week (Figure 13).

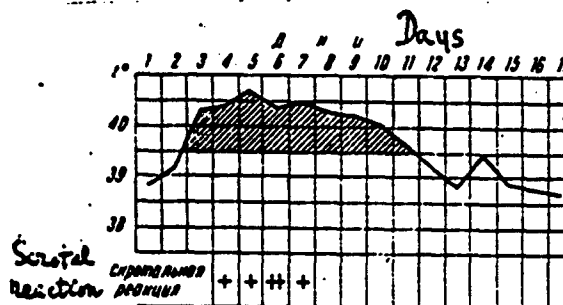


Figure 13. - Fever curve of the guinea pig. Peritoneal rickettsiasis. Inoculation with an egg culture of RPr. Febrile and scrotal reaction.

Autopsy of infected male guinea pigs at the height of infection (4th-5th day), together with a rickettsial periorchitis of purulent or fibropurulent character, reveals the presence of a specific peritonitis with adhesive scanty exudate that covers the peritoneum. In scrapings from the testicular membranes, the classical picture of rickettsiasis is displayed with abundant multiplication of the agent in the mesothelial cytoplasm. But in scrapings from the peritoneum, here are very frequent the characteristic thread-like rickettsiae, once individually, once in mass filling up the mesothelium.

In guinea pigs, peritoneal rickettsiasis is very demonstratively reproduced at intraabdominal inoculation of a suspension made from a mouse lung (see below). With the aid of peritoneal exudate, including scrapings



from the peritoneal coatings, peritoneal rickettsiasis can be passed from pig to pig, but it gets weak already in the next subpassage (P.F. ZDRODOVSKII; P.F. ZDRODOVSKII and E.M. GOLINEVICH, 1948). Peritoneal rickettsiasis in guinea pigs usually runs a benign course, and only rarely ends in death.

The indicated form of peritoneal rickettsiasis in guinea pigs is characteristic for epidemic TF, however, it is not successful even with the related rat TF for which, at similar inoculation, the formation of periorchitis is characteristic, without lesions of the peritoneal coats of the abdominal cavity.

The passage through a TF infection in any of its forms produces permanent homologous immunity in guinea pigs, and usually a cross immunity to the agent of rat TF. Sera of convalescent guinea pigs, in addition to the serological reactions with an antigen made from RPr (agglutination reaction, complement binding reaction, and so on) will display protective properties for not less than 6 months' duration (time of observation--P.F. ZDRODOVSKII, 1948).

In addition to the above described cyclically proceeding febrile reaction, in association with the immunological serum and antiinfective indices, in TF guinea pigs pathological anatomical changes develop which are characteristic for this infection. These changes are in form of nodular lesions of small vessels, especially in the brain.

In agreement with all the above stated material, the guinea pig is a model animal in all laboratories for the experimental reproduction of TF infection.

Rabbits are not very sensitive to RPr and in case of the usual method of parenteral inoculation they respond with an asymptomatic infection of brief viral invasion of the internal organs which is roughly after a 10-day incubation period (CH. NICOLLE and BLAIZOT, 1916; DOERR & PICK, 1919). In case of intratesticular inoculation of rabbits, multiplication of rickettsiae is regularly observed in the testicular tissue for a period of 14 days, which makes possible to pass the infection from testicle to testicle (A.A. GREENFELD, A.I. SEREBRYANAYA and N.V. NEIMER, 1933). Intracutaneous inoculation of rabbits causes a characteristic local lesion with strong inflammatory reaction, terminating in local necrosis (GIROUD). In difference from guinea pigs, all the listed forms of TF infection in rabbits are associated with a positive Weil-Felix reaction with *Proteus* OX<sub>19</sub>.

Together with the indicated, later examinations established that in case of a tracheal inoculation with sufficient dosage of rickettsial suspension (e.g. suspension of a rickettsial lung of an infected mouse) in combination with simultaneous lowering of the temperature with barbiturate anesthesia to 37° or lower, just as in the case of rat rickettsiasis (CASTANEDA, 1939), a specific pneumonia develops in rabbits with ample accumulation of RPr in the lungs 72 hours after inoculation (P.F. ZDRODOVSKII, 1942-1943).

In rabbits, at intraabdominal inoculation of a sufficiently large dosage of RPr (e.g., a suspension made from two rickettsial lungs of an infected mouse), in combination with reduction of temperature, in analogy with the previous experiment, a severe or fatal form of specific peritonitis and periorchitis develops with abundant multiplication of RPr in the cells of the peritoneal mesothelium and in the tunica vaginalis whose serous cavity is filled with purulent exudate containing huge amounts of rickettsiae (P.F. ZDRODOVSKII, 1943).

At simultaneous inoculation of the lung through the trachea and of the abdominal cavity, with the use of the above described methodology, a pulmonary and peritoneal, usually fatal rickettsiasis is produced in rabbits with abundant colonization of the infected cavities with RPr (P.F. ZDRODOVSKII, 1943).

Finally, in case of inoculation of RPr into the anterior chamber of the eye, in rabbits a specific iridocyclitis develops. An external ocular lesion is initially detected a few days after the inoculation in the form of pericorneal injection, and hyperemia of the iris. Further on, the inflammation increases

progressively, spreading to the cornea and the iris, and it ends with the picture of a pannus. Later on, the inflammatory process gradually ceases, and it ends in full recovery with restoration of vision (T.V. BOCHAROVA, 1948 et al).

With the usual methods of parenteral inoculation mice and rats yield an asymptomatic form of TF infection which is getting weaker in mice after 3-4 passages (J. LEGRAT, & JADIN 1932; 1933; et al).

Intranasal inoculation of mice under either anesthesia with a mixture of RPr usually causes a lethal, easily passable pneumonia in them with ample accumulation of rickettsiae in the lungs (DURAND & SPARROW, 1940; M.M. MAEVSKII, 1941, et al), with simultaneous presence of the virus without different rickettsiae in the blood, brain and in all internal organs (M.M. MAEVSKII, 1945). In case of mouse pneumonia especially abundant multiplication of rickettsiae occurs in the septal cells, otherwise the so-called alveolar epithelial cells (A.P. AVTSYN, 1945) which are fully loaded with a huge amount of small rickettsiae. In connection with the abundant accumulation of rickettsiae in the mouse lung, the production of a pulmonary form of TF is the most productive method in them for a mass acquisition of RPr, as this was shown by French and Soviet authors (GIROUD and collaborators; M. MAEVSKII, and collaborators; M.K. KRONTOVSKAYA and collaborators).

In difference from rat TF which is caused by R. mooseri, intraabdominal inoculation of mice with RPr under the usual conditions is without results. More exactly, it causes only an asymptomatic infection. On the contrary, in mice which are preliminarily subjected to x-ray radiation, intraabdominal inoculation causes a fatally running rickettsial peritonitis, easily passable to irradiated mice by way of subsequent intraabdominal subgrafts of the peritoneal exudate (P. LIU, J. SNYDER, J. ENDERS, 1941).

Similar inoculation of benzol treated or irradiated rats with a RPr suspension remains without results in difference from the experiments with R. mooseri inoculation which in such rats produces intensive forms of a specific peritonitis (ZINSSER and CASTANEDA, 1931 - 1932).

Even though rats are very sensitive to TF infection, yet they can be well used for the titration of RPr suspensions by their intraabdominal inoculation with diminishing doses of rickettsial suspensions under the control of complement binding reactions 3-4 weeks after infection. Such tests on rats, compared with guinea pigs (see above), usually give a reduction of titre only by one logarithmic index.

Cotton rats, according to the observations of American authors (SYNDER & ANDERSON, 1942; ANDERSON 1944), show a very high sensitiveness to RPr. Their inoculation succeeds with intracardiac and intracerebral introduction of the virus; moreover, the infection can be easily maintained in passages. Particularly often, they use here intracardiac inoculation with the employment of liver suspensions in passages. In this suspension, we observe the maximum concentration of rickettsiae (it is used as 0.1 - 0.2 ml centrifuged liver suspension, rubbed down in 4-5 ml of bouillon). At an excess dosage of rickettsiae, the TF infection in cotton rats runs as an acute ailment, ending in death in 2-4 to 6-8 days after inoculation. At autopsy of dead animals, the picture of disseminated rickettsiasis is found with maximum concentration of the agent in the brain and liver. At intranasal inoculation, in cotton rats, just as in mice, rickettsial pneumonia is easily reproduced. According to the data of PRICE (1953), in the brain and kidneys of infected cotton rats RPr are preserved up to 5 months, and after a more recent observation of F.I. KRASNIK (1963), for more than 6 months.

For the characteristics of the sensitiveness of cotton rats to TF infection, especially indicative are the observations of FULLER (1953). In parallel experiments of inoculating body lice and cotton rats with identical doses of an egg culture of RPr (in the range of dilutions  $10^{-3}$  - to  $10^{-8}$ , under control of immunological indices, the author revealed an approximate sensitiveness in lice and cotton rats of TF virus. In similar experiments of titrating the brain TF virus of a guinea pig, which was done in our laboratory, it was also shown that the sensitiveness of cotton rats to inoculation with this virus, when the infection in cotton rats is determined by complement binding reaction after a month's period approaches the sensitiveness of body lice (N.G. KOKCHEEVA, & V.A. YABLONSKAYA, 1954).

### CLINICAL PICTURE OF TYPHUS FEVER

The clinical picture of TF was described in great detail in the domestic literature by highly competent specialists in infectious diseases in appropriate manuals and special monographs (K.F. FLEROV, 1914; D.D. PLETNEV, 1920, N.K. ROZENBERG, 1920, 1936; G.A. IVASHENTSEV, 1948; A.M. SEGAL, 1934; N.I. RAGOZA, 1946, 1955; A.F. BILIBIN, and K.F. BUNIN, 1956; K.M. LOBAN, 1960), not to mention then the many journal articles on pertinent partial problems.

Below, for the sake of completeness of exposing the problem, only the most general information is given on the clinical picture of TF whose description is separate for epidemic and for sporadic TF.

#### EPIDEMIC TYPHUS FEVER.

The clinical picture of epidemic TF in its most general outlines can be characterized in the following manner.

The incubation period is usually 10-12 days (with possible variations, by the data of HAMDI, from 5 to 23 days. In a self-inoculation tests made by O.O. MOCHUTKOVSKII the incubation period was 18 days). According to the data of N.K. ROZENBERG (1936), the length of incubation can vary from 8 to 13-14 days. D.D. PLETNEV (1920) pointed out that the incubation period varies more often from 7 to 14 days, with a rarity of the extreme dates (shorter and longer).

The fever is characterized by rapid or slower rise of temperature which in the latter case reaches a maximum by the 3-4th day (Figure 14). In the first period of the disease, the type of fever is steady, while, from the 9th to 11th day on, it has a remittant character. At the height of fever development, the temperature reaches  $40^{\circ}$  -  $41^{\circ}$  and more (Figure 15). The duration of fever, according to the data of L.V. GROMASHEVSKII (1947), varies from 9 to 16 days. Moreover, in the majority of cases (66.1%) it is 12-16 days. Sometimes the febrile period is 9-11 days (15.2%) or, on the contrary, it lasts 17-21 days (17%). But, as the author emphasizes, a length of fever more than 16 days is obviously due to complications.

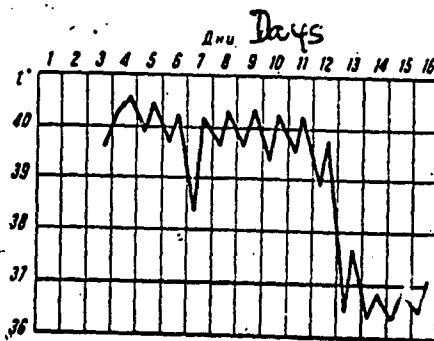


Figure 14. - Fever curve of a patient of epidemic typhus fever (after D.D. PLETNEV, 1920).

The fever ends critically, or with an accelerated lysis (K.F. FLEROV, 1914).

The exanthema (rash) is a characteristic symptom of the disease, and it is absent only in a limited number of cases (in 6% - 8% according to N.K. ROZENBERG). It appears from the 3rd to the 5th day on, reaching its bloom on the 5th-6th day (K.F. FLEROV). At the beginning the rash appears on the chest, spine, abdomen, and then it spreads to the extremities. On the face it is

rarely seen. Sometimes it can be on the palms and soles (K.F. FLEROV, N.K. ROZENBERG). The rash can vary in its character. Beginning in the form of roseolae, it can later take a petechial character. In other cases, it can be a petechial rash from the beginning. Finally, in some usually benign cases, the rash remains roseolar papular. In cases of medium severity it lasts until the 12th to 14th day, and in severe cases, it can remain for some time even after the end of fever (K.F. FLEROV).

The nervous system is damaged constantly, in epidemic TF, not infrequently with marked symptoms of meningo-encephalitis. From the symptoms which refer to the nervous system's lesions we must mention the strong headaches, vertigo, insomnia, different degrees and strength of disturbances of consciousness, and symptoms of hyperesthesia, in serious cases--pareses and paralyzes of the sphincters and fine convulsions and tremor of the muscles.

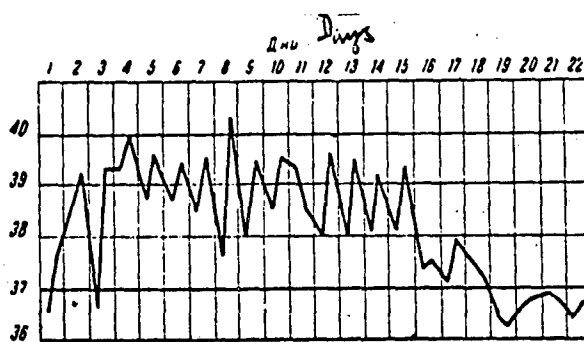


Figure 15. -

The nervous system is damaged in the following order. During the first week, the lesions of the sympathetic system are in the foreground. On the second week, changes are seen in the same and in the oblongata. On the third week, lesions of the brain and the oblongata predominate (G.A. IVASHENTSEV).

The cardio-vascular system is also very actively involved in the process in TF. Specially characteristic for the latter is the disorder of blood circulation which, according to the modern findings, is pathogenetically related to toxic paralytic lesion of the small vessels ("peripheral" heart) (A.P. AVTSYN, 1955). This initial lesion in the vessels subsequently inhibits the function of the vasomotor center in the brain, of the sympathetic sector of the nervous system, and of the suprarenals, which in their turn lead to further deterioration of the blood circulation (K.M. LOBAN, 1960). The disturbance of blood circulation is manifested by hypotonia and increased pulse rate up to 120 per minute which are characteristic for TF, while in severe cases the pulse rate is up to 140-150 beats per minute. In particularly serious cases, all this can lead to a condition of collapse.

In comparison with the vascular apparatus, the changes observed in the heart in TF have only secondary importance (I.V. DAVYDOVSKII).

In the blood, moderate leukocytosis is encountered at the expense of an increase in the number of neutrophils and monocytes with characteristic appearance of Türk cells (up to 8-10%; according to N.K. ROZENBERG).

The spleen is usually enlarged (in 90% of the cases already from the 3rd to 4th day of sickness on; (G.A. IVASHENTSEV).

In the gastrointestinal tract the dry coated tongue is of special interest, with a general tendency of the sick to constipation.

The liver is sometimes enlarged. In the lungs, together with the symptoms of bronchitis, bronchopneumonia is not infrequently detected.

In the kidneys, albuminuria is often noted. In a later period, the importance of uremia is emphasized which arises in connection with renal insufficiency. The development of the latter is evidently the earliest indication of a further severe course of the disease. The cause of renal failure is the rickettsial lesion of renal vessels and the fall in arterial blood pressure (SNYDER, 1947).

Among the complications of TF, we mention: bedsores, abscesses and cellulitides, phlebitis and thrombophlebitis, parotitis, otitis, pneumonia, nephritis.

#### CLINICAL VARIANTS OF EPIDEMIC TYPHUS FEVER.

The main and most characteristic symptoms of TF which are outlined above, may considerably vary in their strength and severity. In accordance with this, the clinicians used to distinguish mild, moderately severe, severe or very severe forms in TF. According to the data of V.A. BARYKIN (1932), the indicated forms were observed in different epidemics in the following percentages as related to the total number of recorded patients. Most frequently, the disease is met with at its average severity, which is 70%-80%. The mild form is observed within the range of 10%-25%, and the severe within the range of 5% to 10%. The actual diversity in the clinical severity of epidemic TF is particularly clearly illustrated by extreme figures in the length of febrile period. Thus, according to the data of D.D. PLETNEV (1920), the length of the latter can vary from 3 to 22 days with absence of complications. In analogy with this, K.M. LOBAN (1960), who refers to the findings of M.P. KIREEV, K.F. FLEROV and others, points at the length of fever from 2 to 21-22 days.

In the group of severe and very severe forms of TF, according to the predominance of some syndrome, we distinguish:

- a. a hyperpyretic type with a temperature elevation up to 41°-42°;
- b. a hemorrhagic type with very marked petechial rashes which take the nature of actual hemorrhages;
- c. a nervous type with especially marked lesion of the central nervous system;
- d. a fulminating type at which death will occur already after 2-5 days (D.D. PLETNEV, 1920).

In connection with the above indicated, we cannot pass by with silence the hypothesis of some, especially English authors (C. STUART-HARRIS et al, 1946) on the different malignancy of TF at different places. Thus, according to his data, in the years of the Second World War (1942-1943), the TF outbreaks of North Africa and Central Asia were distinguished by particular malignancy, where among the French and English the TF lethality was equal to 32% - 35.8% (the statistics refer to 591 cases).

From the viewpoint of epidemiology and control measures, mild forms of epidemic TF are of special interest. Among the earlier authors, they figured under the name of "febris exanthematica levis", and "levissima", including the same category various forms of a mild course of TF infection,

especially the abortive, ambulatory, and obliterated forms in general. These forms are especially peculiar to children.

In the mild forms of TF, its characteristic symptoms (fever, rash, intoxication symptoms, neural and vascular lesions) are usually present, but all of them are manifested in an attenuated form.

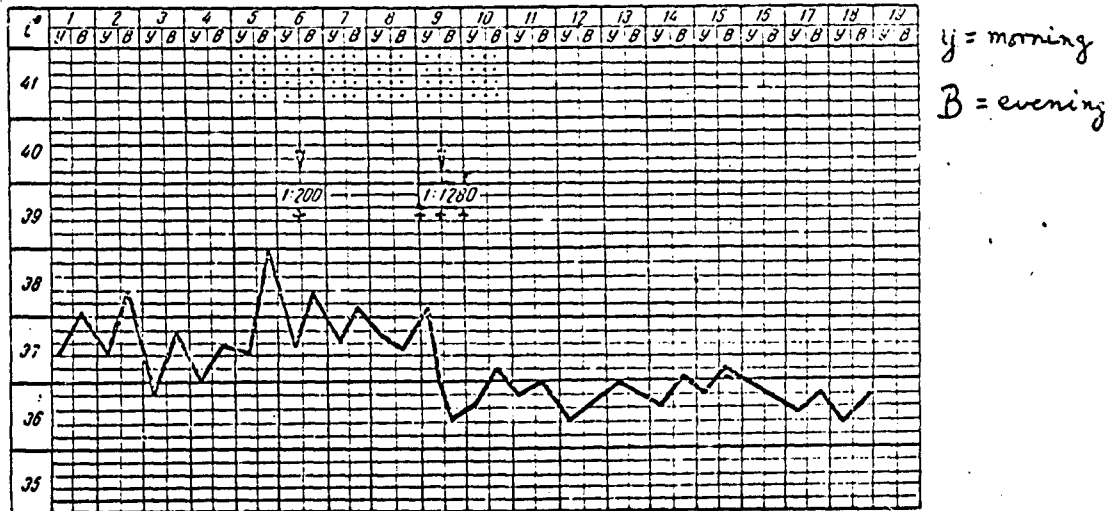


Figure 16. - Fever curve of a patient with atypical form of epidemic typhus fever (after S.S. VISKOVSKII) (quoted after N.I. RAGOZA, 1955).

Thus, the febrile period, with elevation of the temperature usually within 38°, will last 7-9 days, and only rarely 10-12 days (Figures 16 and 17). The predominant forms of the rash are roseolae. Headache and insomnia are marked, but other symptoms of the nervous

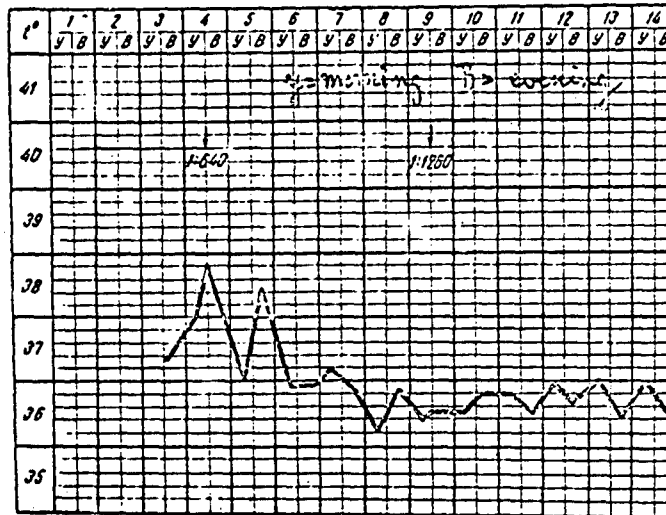


Figure 17. - Fever curve of a patient with atypical form of epidemic typhus fever (after S.V. VISKOVSKII) (quoted after N.I. RAGOZA, 1955).

system are absent or attenuated. The symptoms of general intoxication are slight and the typhous condition is usually absent, with maintenance or slight obfuscation of the consciousness. Hypotonia is absent, or weakly marked, with a drop in the systolic pressure by 10-15 mm Hg. These are the general outlines of the picture of a mild case of epidemic TF as described by K.M. LOBAN (1960), from the clinic of A.F. BILIBIN.

We should add to the above outline that D.D. PLETNEV (1920), in describing mild TF, points out the frequency of catarrh of the upper respiratory pathways which is found in this type of TF in form of laryngotracheitis, sometimes with associated bronchitis, a sign for which earlier authors separated this variety under the term of "catarrhal typhus fever".

Among the described forms, the mildest, with shortened febrile course and slightly marked roseolas, can be carried ambulantly by the patient, in correspondence with which they are distinguished under the term "ambulatory typhus fever" (D.D. PLETNEV, 1920).

French authors describe different variants of the mildest clinical forms of TF infection under the name "formes frustes" of typhus fever. DOPTER and de LAVERGNE (1927), in their well-known treatise on epidemiology, in the section on TF, present numerous illustrative "formes frustes" of TF, described by specialists who studied the epidemic and endemo-epidemic outbreaks of this infection in various countries. Let us give some of these illustrations.

During the 1916 TF epidemic in Romania, CANTACUZENE, the well-known microbiologist and epidemiologist (1920), observed many cases of TF in which "weak rise in temperature, preceded sometimes by non-distinguishable rash, slight headache" was noticed. Moreover, the patients continued their occupation with the sensation of slight indisposition only. Surprised by these observations, the author undertook a special study of the outbreak of TF in one of the villages near the town of Yassi whose population lived in poverty and overcrowding. Here, the author not infrequently observed the following picture. With the presence of 1-2 cases of classical TF in a family, in the other members of the same family at the same time and further on (after several days) he noted elevation of the temperature up to  $37.5^{\circ}$  -  $38.2^{\circ}$ , slight headache, individual roseolar papular rash with generally good feeling, without any special complaint of the patients who remained ambulant. In one case, CANTACUZENE observed a similar case in a physician who complained only of easy fatigability with a temperature up to  $37.4^{\circ}$ , with a few individual rashes on the skin.

By analogy with what was said, PETROVICH (1921) described many cases of formes frustes and forms of benign course of TF which he observed in 1915 during the well-known TF epidemic in Serbia.

Ed. SERGENT, FOLEY and VIALATTE (1921) in their turn describe the spread of cases of an atypical course of TF among Algerian Arabs. On their part, CH. NICOLLE, CONSEIL and CONOR (1911) emphasized the special frequency of atypical, hardly diagnosed forms of TF in children in whom the disease is reduced to a slight fever, coated tongue and mild malaise with or without a rash. (Figure 18).

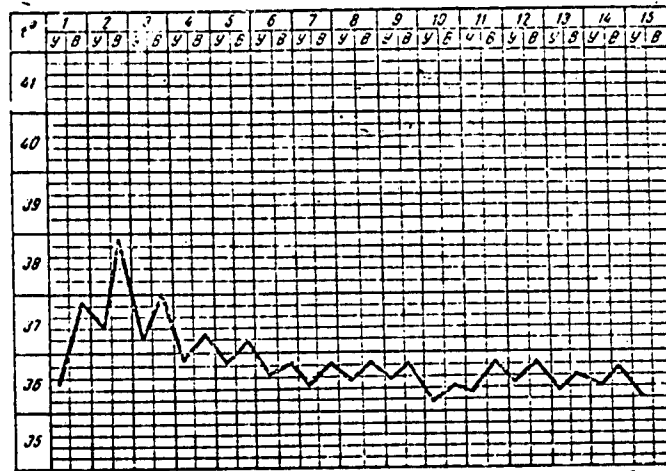


Figure 18. - Fever curve in an atypical form of tphyus fever in a child (after M.G. DANILEVICH).  
Quoted after N.I. RAGOZA (1955).

Such is the picture and the spreading ability of atypical forms of epidemic TF according to the findings of foreign authors. These forms fully correspond with the above mentioned variant of a mild course of this infection as described by domestic authors.

On the basis of many researchers of our laboratory, conducted with the aid of modern sero-immunological methods, a large spread of atypical forms is unquestionable during TF outbreaks.

In conclusion, it should be mentioned that, together with the atypical benign forms of TF infection, in people who are strongly exhausted and whose reactivity is reduced, the lethal forms of TF can also take their course without much manifestation of a clinical picture. These forms of "asthenic" TF, with lethal outcome, produce striking deaths due to physiological exhaustion (PETROVICH, 1921).

Let us say now something on the question of asymptomatic forms in epidemic TF which according to the established tradition, continue to cause a very critical attitude among many domestic specialists of infectious diseases and epidemiologists. L.V. GROMASHEVSKII, one of the most active opponents of the existence of asymptomatic forms, was absolutely right when he announced in his time (1947) in the manual on special epidemiology that if there were obliterated forms in TF, this would beforehand decide the question in a favorable sense about the possibility of the "asymptomatic" forms of infection. But the question about obliterated forms in TF can be scarcely doubted at the present time<sup>1)</sup> which, in agreement with the quoted suggestion of L.V. GROMASHEVSKII, actually decides beforehand the existence of asymptomatic forms in TF infection.

As to asymptomatic forms, we say at present that they are not only trustworthy assumptions, but we have also direct observations at our disposal which are accurately based upon modern methods of investigation. We discuss this question in more details in the section on epidemiology of TF. Here we limit ourselves only to pointing out the well documented observations of the Romanian (COMBIESCU, G. ZARNEA et al, 1957-1959), and partly Polish (J. KOSTRZEWSKI,

FOOTNOTE: <sup>1)</sup> For additional information on the obliterated forms in the light of modern findings see the section on epidemiology.



E. WOJCECHOWSKI, 1959), and American (FOX, MONTOYA, et al, 1959) authors who are quoted in detail in the mentioned section on epidemiology. Let us also give pertinent example on the experiences in our laboratory.

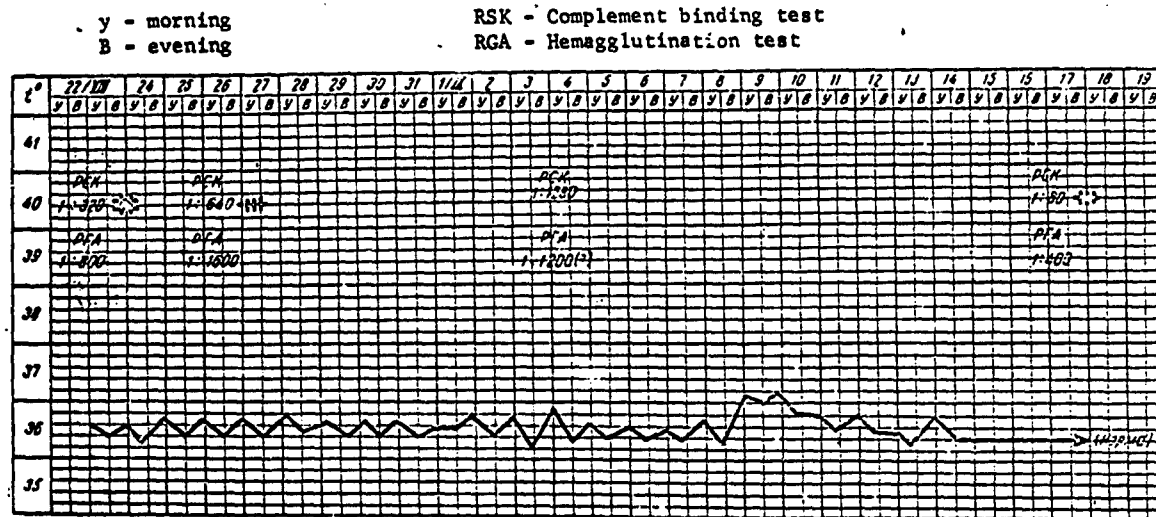


Figure 19. - Subclinical (symptomless) form of typhus fever infection (after G.R. GAZIZOVA).

In one of the collectives a case of TF was detected which had a well marked clinical picture while the patient had diagnostic titres of specific serological reactions (complement binding reaction which gave the following changing values in titres 1:40 ---1:320---1:320, with positive hemagglutination reaction at titres 0---1:12 800 ---1:200).

As to this case after a week, in the collective a general non-immunological examination was carried out, at which in the completely healthy citizen, G., 29 years of age, a positive CBR was found at a titre of 1:320, and a HAR at 1:800. This citizen was subjected to strict clinical observation for a month, with daily double measurement of his temperature, with progress sero-immunological examination which gave the following results:

DATE OF EXAMINATION	COMPLEMENT BINDING REACTION	HEMAGGLUTINATION REACTION
21/VIII	1:320++	1:800
25/VIII	1:1280++	1:1600
4/IX	1:640+++	1:200 (?)
18/IX	1:80+++	1:400

During the whole period of clinical observation, in citizen G., the temperature did not increase even once, and he had a generally good sensation of a fully healthy man (Figure 19).

The quoted observations, completed by experimental serologist G.R. GAZIZOVA with subsequent verification of the sera in our laboratory, in our opinion unquestionably established the case from the point of view of modern requirements as one of an asymptomatic form of TF infection, many times observed by Romanian investigators.

Ultimately, in spite of the widely spread ideas in our country which took shape at a time when there were no required methods of sero-immunological investigations to permit with reliability the exposure of asymptomatic forms of TF infection (observations of S. RAMZIN, V.A. BARYKIN, and collaborators et al), at present this question should not cause any doubt. In other words, in case of TF, as also in other viral and bacterial infections, contamination with RPr can cause all forms of clinically manifested and non-manifested infections in the range beginning from serious and more serious forms and ending with asymptomatic forms.

As a supplement to what was said about the atypical and subclinical (asymptomatic) forms of TF infection, let us quote from our archives similar findings of A. TURSUNOV (1953) who examined the progress of CBR and HAR for 9-16 months in a group of 172 sero-negative healthy inhabitants of one of the country districts afflicted with epidemic TF. First, these serological examinations coincided with the presence of TF outbreaks on the background of marked pediculosis. The quoted data are presented in Table 8.

PROGRESSIVE MOVEMENT OF THE TITRES OF CBR AND HAR IN HEALTHY  
PEOPLE IN A FOCUS OF EPIDEMIC TYPHUS FEVER

Examination	Total No. of Examinees	CBR titre						
		Neg.	1:10	1:20	1:40	1:80	1:160	all +
I	172	172	0	0	0	0	0	0
II (9-16 months after)	172	139	7	6	17	1	2	33
-----		HAR titre						
		Neg.	1:250	1:500	1:1000	1:2000	1:4000	all +
I		172	0	0	0	0	0	0
II (9-16 months after)		161	7	1	1	0	2	11

TABLE 8  
-----

As it can be seen from Table 8, 16 months after the start of examinations, out of 172 sero-negative persons 33, or 19.1% became positive for CBR and RPr antigen, including two cases with the presence of a diagnostic titre of 1:160. At the same time, in a parallel examination with HAR, among the same 172 persons who were originally negative, in eleven persons the HAR was found positive with RPr antigen moreover, in three persons the titre was in the order of 1:1000 - 1:4000, without any doubt to the active condition of TF infection.

In spite of the certainty of the transmission of TF infection by people who gave a positive CBR and HAR, especially by people with high indices of these reactions, none of them had any clinical symptoms of the disease. Thus, in this section of investigations, the existence of atypical and asymptomatic forms of TF infection was once more corroborated in TF in an undoubted form.

The quoted observations simultaneously clearly illustrate the production of positive serological reactions in healthy people who lack any TF ailment, as this is exposed at the study of the immunological structure of the population in localities which were previously subjected to TF outbreaks (see below).

#### SPORADIC TYPHUS FEVER OR BRILL'S DISEASE.

Under sporadic TF we now understand a clinical epidemiological variety of TF which is etiologically identical with epidemic TF, but differs from it by a number of peculiar features, including the episodic character of individual sickness cases, usually in the absence of pediculosis, and with an inability to recognize objectively the probable source of infection. There is a benign course of the disease, relative difficulty in isolating RPr from the patient's blood, presence of early appearance and strong manifestation of serological reactions in the patients with the use of specific antigens prepared from RPr, but with frequent absence of the Weil-Felix reaction with *Proteus* OX<sub>19</sub>, predominant susceptibility of adults and older groups of inhabitants, absence of marked seasonal character which is a characteristic for epidemic TF.

Sporadic TF is recorded among people who in the past were subjected to outbreaks of epidemic TF, including persons who emigrated from localities where TF was at home into districts which were free of the infection.

In the above quoted characteristic, sporadic TF is identical with recurrent TF which can be identified in the nomenclature of western authors and of the International Nomenclature of Diseases as Brill's disease. It should be noted, however, that sporadic TF with the above quoted characteristics is also recorded among people who deny any previous TF in the past. But this denial does not exclude an actual second (recurrent) TF infection in them, since, as we have seen, epidemic TF can have an atypical course and in this case it often remains unrecognizable---a circumstance which requires correction of the anamnestic data, as this is especially corroborated by the results of retrospective detection of TF infection with the aid of serological examinations of the population (see the pertinent section).

In every case, such an idea which objectively widens the category of people who are sick with sporadic TF as a recurrent disease, appears fully probable, and not without foundation, it is emphasized by several authors (K.N. TOKAREVICH, 1958), completely corresponding to modern findings on the spread of atypical and subclinical forms of TF infection. Hence, to the denomination "sporadic" TF more preferable is the term of "recurrent" (or second) TF.

As to the clinic of sporadic TF <sup>1)</sup>, frequently or in the majority of cases, the clinical picture corresponds with the course of mild forms of epidemic TF, preserving at the same time usually the outstanding and characteristic symptoms of the latter, as this is emphasized by K.M. LOBAN (1960). This symptomatology, relating to the injuries of the vascular and nervous system, is not infrequently weakened to some extent. Here, atypical forms, peculiar also to epidemic TF, are more frequently met with in sporadic TF; obviously on this account, it is not infrequently recorded under mistaken diagnoses (grippe, catarrh of the upper respiratory tract, paratyphoid, pneumonia, indefinite obscure febrile conditions, and so on), and it is recognized in such cases only with the aid of serological tests.

The febrile period in sporadic TF is equal to 8-10-11 days as an average, but it can be also shorter. According to the summarized data of K.M. LOBAN, the fever counts 9-11 days with variations of 2-3 days in both directions.

<sup>1)</sup> FOOTNOTE: The clinical characteristic of sporadic TF is basically given according to its monographic description by K.M. LOBAN (1960), which contains the pertinent extensive literature material and the personal observations of the author).

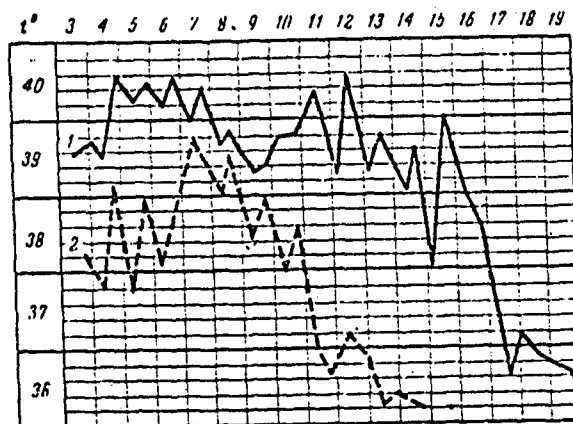


Figure 20. - Fever curve of patients with primary (1) TF in the son and secondary (2) TF in the father (from the archives of S.M. KULAGIN).

In agreement with this, according to the data of M.A. KULEMINA and K.P. KUZNETSOVA, for 264 patients with sporadic TF the length of fever was scattered at the following rate: 5-8 days...20%; 9 days...18.8%; 10-11 days...40%; and 12-17 days...21.2%. According to the summary data of A.P. ASTAKHOVA, M.V. MATVEEVA and G.P. SETUNOVA, among 153 patients with sporadic TF the same rates were recorded in this forms: 7-9 days...31.5%; 10-12 days...66%; and 13-16 days...6.9% (see also Figure 20).

Finally, according to the observations of Romanian authors (COMBIESCU et al, 1957), the length of the febrile period was 4-6 days in 6 patients; 7-9 days in 48; 10-11 days in 28, and 12-14 days in 18 patients.

If the above given figures which characterize the length of fever in sporadic TF are compared with similar data for epidemic TF, as they were presented by L.V. GROMASHEVSKII (1947), then an essential difference is found (e.g., for L.V. GROMASHEVSKII the length of fever for 4-5-8 days is absent, while the 12-16 days fever becomes 66.1%).

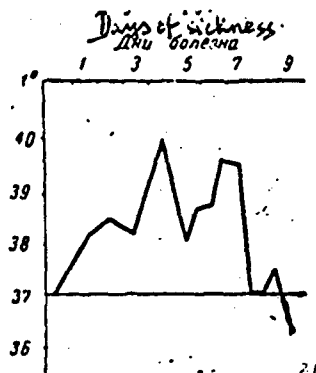


Figure 21. - Fever curve of a patient with sporadic (recurrent) TF. On the 8th day the CBR was 1:800, the toxin neutralization reaction 1:2048. On the 14th day the CBR was 1:6400, the toxin neutralization reaction 1:8192. Original diagnosis: abdominal typhoid (K.N. TOKAREVICH, and collaborators).

The difference is especially clearly illustrated by comparing the data for the fever length in sporadic and epidemic TF (after L.P. GROMASHEVSKII) according to the observations of G.R. GAZIZOVA.

In approximately two-thirds of the patients with sporadic TF, the skin rash is abundant; it is of roseolar petechial character, while in one-third of the patients it is not abundant, and it keeps its roseolar character. The rash is absent in 8-15% of the cases, just as in the epidemic form.

The cardiovascular system is disturbed, but to a lesser degree than in epidemic TF. Tachycardia occurs in 22-32% of the patients (M.L. LOBAN). Hypotonia can be observed to some extent, with some steadiness, but it practically almost never assumes an emergency character.

On the part of the nervous system, the most constant symptom is a markedly expressed headache. Typhous condition of some severity is usual. Psychic disturbances, peculiar to epidemic TF, are rarely met with in the sporadic forms, and they are less marked. There are also observed respiratory troubles peculiar to TF. Frequently the skin is hyperesthetic.

In comparison with epidemic TF, in sporadic TF, complications are much less frequently met with, and this "is one of the most important peculiarities of sporadic TF", as M.K. LOBAN emphasizes.

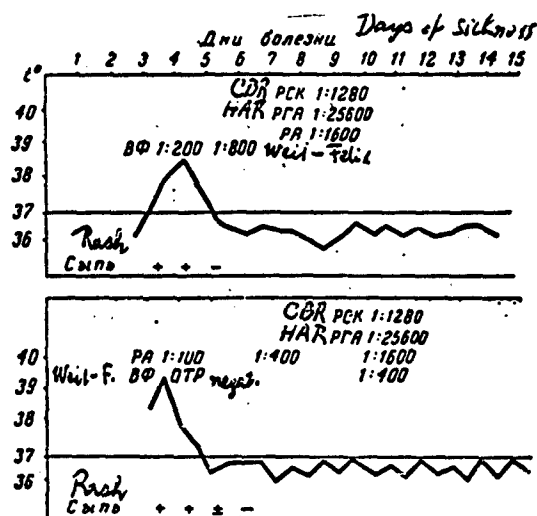


Figure 22. - Fever curve of patients with sporadic typhus fever (after G.R. GAZIZOVA).

In sporadic TF the prognosis is favorable. Usually it ends with recovery. By the majority of authors, fatality is recorded at 0.6 - 0.8 to 1.68%.

As a supplement to the above discussion on the peculiarities of the clinical picture of the contemporary forms of sporadic TF, we give hereto pertinent data published by M.M. FIGURINA and A.N. SEMENOVA, which gives long range observations on these authors on the material of the Botkin Hospital in Leningrad where non-epidemic and familial foci of typhus and only individual sickness cases were recorded already earlier as a rule, in absence of pediculosis in the patients and when the source of their infection was unknown.

In their review, M.M. FIGURINA and A.N. SEMENOVA emphasize the following peculiarities of the TF cases which they observed during recent years.

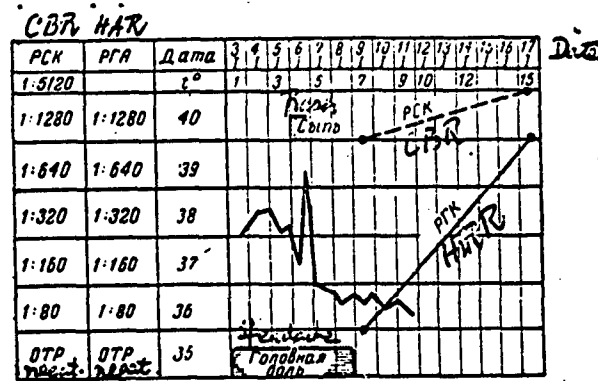


Figure 23. - Fever curve of a patient with sporadic (recurrent) typhus fever.

CBR...complement binding reaction;  
HAR...hemagglutination reaction.

1. In the majority of cases, the sickness occurred in people over 45 years of age who made 59.3% of all the recorded patients.

2. Among the sick people more than 70% of the second (recurrent) TF were recorded.

3. In 60% of the patients, the WEIL-FELIX reaction gave negative results, with 100% positive serological reactions to antigens made from RPr (*Rickettsia prowazekii*).

4. The clinical picture of the contemporary forms of sporadic TF is sharply distinguished from the classical (epidemic) TF by the mildness of course, and the abatement of all the symptoms, to wit:

a. the febrile period was much shorter with an average duration of 6-8 days, with an increasing tendency to accumulation of 7-day sickness cases.

b. the symptoms of intoxication are much weaker in the patients, with preserving the hypotonia characteristic for TF infections.

c. As a characteristic which is related to the elderly age of TF patients, the authors point out their tendency to develop vascular complications.

Such are the general outlines of the clinical picture of sporadic TF. As we see, retaining the typical features of TF, sporadic TF has a course in a considerably milder form compared to epidemic TF. Here it should be kept in mind that such a mild course of TF infection in a given case is observed chiefly in people of elderly age, i.e., in an age group specially unfortunate also with regard to the high lethality of those who are passing through the epidemic form of TF.

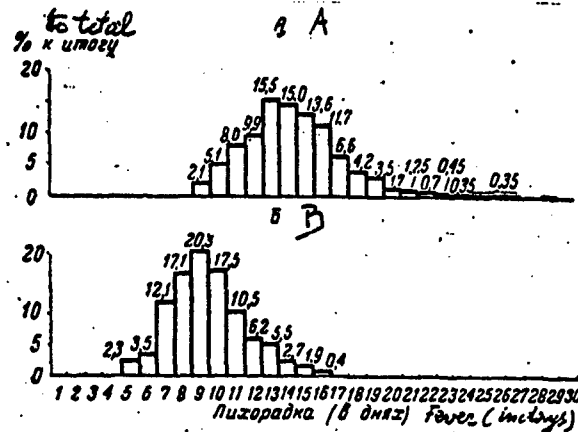


Figure 24. - Comparison of the length of typhus fever.

A...in the epidemic forms (L.V. GROMASHEVSKII and collaborators, 1947), and  
B...in the sporadic forms (G.R. GAZIZOVA)

In conclusion let us note that, due to the spread of mild forms with the existence of different variants of mild course (atypical, ambulatory, obliterated forms), the clinical picture of sporadic TF is characterized by a certain polymorphism which obviously makes also difficult its diagnosis, not infrequently---as already noted above---accessible only to sero-immunological methods of investigation. At the same time, the accumulation of cases of mild or easy course also determines the "peculiarity" of clinical picture in sporadic TF, but this peculiarity is only apparent, since the same types of mild picture of the disease, as we have seen, have been known long ago in epidemic typhus fever, too.

PATHOMORPHOLOGY AND PATHOLOGY OF TYPHUS FEVER

As it has been known since the times of the finds of N.A. ALFEEVSKII (1914) and FRAENKEL (1913-1915), with regard to pathomorphology, TF is considered a disease with predominant lesion of the vascular system (I.V. DAVYDOVSKII, 1920).

Such an idea is fully justified by the morphological documentation of TF ailments, since the desquamative proliferative processes in the small vessels which lead to the formation of vascular granulomas characteristic for this infection, described by D.V. POPOV (1875) in his time in the brain, in combination with destructive thrombotic changes compose almost the whole morphological symptom complex of TF.

At the same time the location of lesions near the vascular system fully corresponds also to the microbiology of TF, since in TF the RPr have a selective localization in the cells of the vascular endothelium.

In view of this, the description of the pathological anatomy of TF in all its respects is best started exactly with the lesions of the vascular system, classically presented in the generally known monograph of I.V. DAVYDOVSKII "Pathological Anatomy and Pathology of Typhus Fever" (1920-1922).

As I.V. DAVYDOVSKII indicated in the quoted monograph, in TF in the course of vessels a double sort of process is observed: ---desquamative proliferative and destructive thrombotic.

DESQUAMATIVE PROLIFERATIVE PROCESSES.

In correspondence with the colonization of the endothelium of vessels with RPr, it is natural that the disease of vessels begins with endothelial changes in the form of their marked swelling in the affected sectors of small vascular branches and capillaries. The swollen cells of the endothelium at the same time show active multiplication, frequently with detachment into the lumen of vessels. As a result, inside the affected parts of vessels, whole colonies of multiplied endothelial cells are formed, which sometimes leads to the formation of peculiar "proliferative" thrombi.

The proliferative process is not limited to the endothelium, spreading on the one hand to the cells of the intima, and on the other hand to the adventitial elements, or the so-called perithelium. The endovascular and perivascular proliferation here flows together in the small vessels into a common conglomerate, keeping the independence of perivascular proliferation only along the course of relatively larger vessels.

As a result of the proliferation of endo-perithelium along the course of affected vessels, granulomas are also formed which are characteristic for TF, and are called Popov's nodules. According to the expression of I.V. DAVYDOVSKII, they "run as a red thread throughout the entire histology of typhus fever".

Arising from tissues of mesenchymal origin, the mentioned granulomas have the aspect of a homogenous type of miliary nodules which are situated along the course of precapillaries and capillaries, being the products of endo-perithelial proliferation. In this form of focal granulomatosis, these isolated nodules are met with in TF in all systems and organs, with the exception of the liver, lymph nodes, spleen and bone marrow.



At the formation in an ectodermal medium in the parenchyma of the nervous system, the endo-perithelial granulomas are sufficiently hedged in with a peripheral zone of proliferating elements of the neuroglia. As a result, "glial granulomatosis" of the vessels is formed in the brain in TF.

In both forms of granulomatosis, at the site of development of the nodules, the structure of small vessels is obliterated, and they are sort of assimilated into the affected parts.

The described endo-perithelial nodules are composed of lymphoid and epitheloid types of polyblasts tightly adjourning each other, with markedly polymorphic nuclei. In case of cerebral nodules, the endo-perithelial proliferation is forming only the nucleus of a granuloma.

In addition to the above described nodular granulomas which are typical for TF, in this infection we can also encounter perivascular "plasma cell" and "lymphoid" sheaths (cuffs) arranged chiefly along the course of veins, and formed by proliferating perivascular cellular elements. In presence of "sheaths", the permeability of vessels is preserved.

By their topography in regard to the vessels, the above described proliferative processes can be intravascular (frequent form), intramural (rare form) and perivascular (the most comprehensive group).

#### DESTRUCTIVE THROMBOTIC PROCESSES.

Destructive processes along the course of the vascular system, as constant in TF as the cellular proliferations, are also manifested in symptoms of necrobiosis of different elements of the vascular wall.

In TF, the most frequent are the phenomena of necrobiosis in the endothelium, chiefly of small vessels and somewhat less often of larger vessels. Starting from the intima, the necrobiotic process can spread to the middle and external sheath, embracing all their circumference in the affection of small vessels and limited to a segmental lesion in the larger vessels. Finally, the destruction can also spread to the cellular proliferations with necrosis of granulomas and perivascular sheaths. In connection with the destructive changes on the part of the intima in the affected sectors of the vascular system, not infrequently we find thrombi which are parietal, varrucous, or obtirating.

As a result of proliferation, destruction and thrombosis, a focal obliteration and deformation of different severity is obtained, in the vessels, but with full restoration of the blood circulation with their reversible involution without any particular defect in the structure of vessels.

From the changes of individual organs, the lesions of the brain should be specially mentioned in TF. The here pertinent changes are characterized as acute non-purulent disseminated myeloencephalitis whose basis is "granulomatosis" of small vessels in presence of a partial degeneration of ganglion cells. Here, the encephalitis is usually combined with acute serous meningitis.

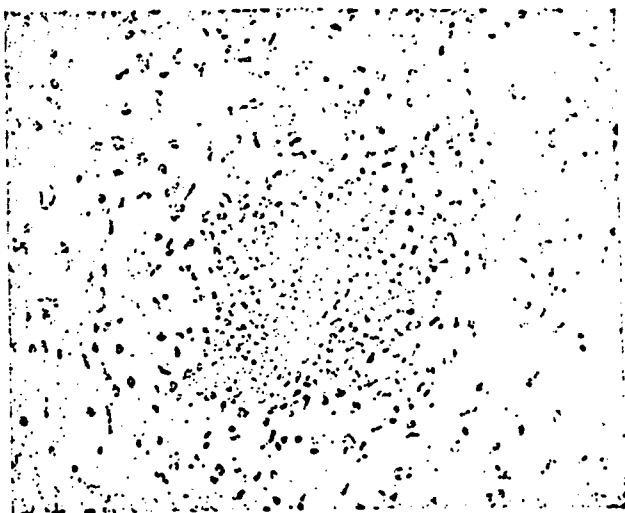


Figure 25. - Typhus fever nodule in the medulla oblongata (photo of A.P. AVTSYN)

Among the vascular lesions, verrucous endovasculitis, destructive and proliferative thrombo-vasculitis, proliferative perivasculitis and plasma-cell sheaths stand out especially characteristically. The lesions along the vessels are fixed to the gray matter of the brain. They can be observed over its entire extent, yet the lesions of maximum intensity and extensiveness are formed in the medulla oblongata (Figure 25).

A similar process affects also the sympathetic nervous system, especially its ganglia.

An involution of the lesions in the nervous system occurs rather slowly.

The lesions of the heart are also very characteristic for TF in the form of acute interstitial myocarditis. As I.V. DAVYDOVSKII states, "every case of TF (with rare exception) is accompanied by an acute interstitial myocarditis". In the picture of the latter, we differentiate on the one hand the formation of miliary nodular granulomas along the capillaries (myocarditis nodosa), and on the other hand the formation of foci of plasma cell infiltrations and sheaths (myocarditis plasmocellularis).

Finally, with relatively rare exceptions, TF is associated with an exanthema or rash on the skin which is characteristic for it. This rash, first roseolar or roseolo-papular, undergoes a petechial transformation in typical cases.

The pathomorphology of the TF exanthema is essentially a lesion of the vascular system typical for TF, at which the vascular system here presents all diversities of its forms.

Together with the vascular lesions in the area of cutaneous exanthema there can be disturbances of blood circulation and infiltrative exudative processes, with the development of hyperemia, edema, extravasation, and so on. Sometimes coagulation necrosis of some extent can be also observed in the epidermis.

Such is the general picture of pathomorphological changes in TF.

As to the complications of TF, in addition to the extravasations of different localization, we should mention the subsequent thrombophlebitides and the gangrene of soft parts, most frequently seen on the extremities, as well as cases of parotitis, cellulitis, septicopyemia, related to secondary infection due to porosity of the affected vessels. In the lungs bronchopneumonias are not infrequently formed.

#### EXPERIMENTAL TYPHUS FEVER INFECTION IN ANIMALS.

Just as in man, in guinea pigs also, which are the basic experimental model for this infection, in experimental TF the pathological anatomical changes are fixed to vascular system with their specially characteristic localization in the brain which is therefore the most common object of investigations.

According to the collected data of I.V. DAVYDOVSKY (1922), on the part of the vascular system the changes in guinea pigs on the one hand consist of destructive thrombotic lesions of small arterioles and capillaries, while on the other hand they consist of proliferations of endo-perithelial type which, together with destructive thrombovasculitis, form the most characteristic histopathological complex of the infection (TF nodules).

In case of peritoneal TF infection, in the inoculated guinea pigs marked exudative processes are noticed in the peritoneum with the presence of exudative proliferative changes along the course of the vascular system of the testicles and an exudate, which is rich in cellular elements, in the cavity of the tunica vaginalis of testicles in case of associated periorchitis.

According to the data of I.V. DAVYDOVSKII, on the part of the vascular system in TF in guinea pigs the changes are essentially similar to those of man, yet they differ from the latter only in two respects: -- compared with man, in guinea pigs the proliferative changes of vessels predominate over the destructive changes, and the granulomatosis in the medulla oblongata which is particularly characteristic for man is absent.

We have still to say a few words on the pathomorphological characteristics of TF bronchopneumonia in mice, which can be reproduced in them with intranasal inoculation of RPr.

According to the data of A.P. AVTSYN (1948), in the development of this rickettsial pneumonia in mice, four stages of primary irritation are distinguished which are characterized by swelling of the alveolar epithelium in absence of vascular reaction (first hours); stage of vascular reaction with diffuse hyperemia and symptoms of diapedesis of red cells (15-24 hours); stage of formation of specific foci (36-42 hours), and the stage of necrotization of the exudate, corresponding to the maximum accumulation of RPr in the lungs (66-90 hours).

According to the histopathological researches of A.P. AVTSYN (1948), in mice the action of the toxic factor of RPr is angioparalytic. It is manifested in a disorganization of blood circulation, mostly in the brain; moreover, the rickettsial toxin evidently acts upon the vascular wall of capillaries and arterioles. The greatest degree of the disorganization of cerebral circulation finds its expression in a capillary stasis in the brain, which is also a direct cause of death of mice in case of intoxication.

PATHOGENESIS OF TYPHUS FEVER.

As it is well known, for TF just as also for other rickettsias, in addition to the marked symptoms of intoxication, particularly characteristic is the failure of the peripheral vascular heart. Therefore, in such cases the most frequent cause of death is vascular collapse. The systemic organic lesion of vessels which is peculiar to TF can be also the cause of severe disorders in the blood circulation, but they alone cannot explain the failure of the peripheral heart which is evidently related to a functional disturbance of vessels. This double, organic and functional disturbance of the vessels, according to I.V. DAVYDOVSKII, is the "center of gravity" of TF sickness. The pathogenesis of the organic lesions of vessels is clear, since the infective process in TF is related to the colonization of toxigenic rickettsiae in the endothelium of vessels.

As to the functional disturbance of vessels, which is evidently related to a lesion of the central and sympathetic nervous system, including the chromaffine system (lesion of the suprarenals), they evidently originate first of all under the effect of rickettsial intoxication which, according to the correct statement of A.P. AVTSYN (1954), is the outstanding factor in the pathogenesis of TF in general.

By studying more accurately the concept of toxic pathogenesis in TF, and relying upon the study of 212 autopsies of TF patients who died at different periods of their ailment, A.P. AVTSYN came to the following conclusions:

In the first week of sickness the cause of death in patients is the specific intoxication whose clearest morphological expression is the severe disturbance of blood circulation in the central nervous system, including congestion of the brain (partly, paralytic hyperemia), with absence of its nodular lesions.

According to the data of the author, the same specific intoxication is also the main cause of death on the second week of the disease, when, together with the formation of nodules in the brain in 98% of the cases, a disturbance in cerebral blood circulation in the form of hyperemia, stasis and capillary thrombosis, especially in the nuclei of the brain stem and in the gray matter of the cerebral cortex, remains an integral part of the histopathological picture of TF in this period.

In difference from the indicated picture, in the third week of TF, the majority of patients die from complications, especially in the form of pneumonias, with constant presence of ripe nodules in the brain.

Various kinds of complications, especially sepsis and pneumonia, as the author states, are also the most frequent causes of death of the patients in the fourth week of sickness, when in the brain regressive nodules are found which are characteristic for this period.

Thus, emphasizing the decisive importance of intoxication in the pathogenesis of TF, A.V. AVTSYN comes to the conclusion that the nodules themselves in the central nervous system, and, especially in the medulla oblongata, are not the cause, but the sequelae of the disturbance of blood circulation under the effect of intoxication.

Finally, it should be mentioned that Soviet authors, headed by I.V. DAVYDOVSKII, sufficiently established also the share of allergic factors in the pathogenesis which find reflection in vascular lesions, and are especially demonstratively illustrated by the presence of strongly marked allergic skin reactions in patients and in convalescents which develop on introduction of a rickettsial antigen.

These are the main features of the pathology and pathogenesis of TF in the light of modern findings.

The main cause of fatality in TF is the general intoxication with severe lesion of the nervous and cardio-vascular system. Some authors make attempts to make more precise the cause of death, by distinguishing particularly death, mostly related to lesion of the brain with symptoms of meningo-encephalitis (45%), or to lesion of the cardio-vascular system with symptoms of collapse (about 20%), or even to combined lesion of the brain and cardio-vascular system (about 30%). Rather frequent causes of death are different complications among which pneumonia occupies the first place (quoted by K.M. LOBAN, 1960).

In TF the indices of fatality, as we have already mentioned above, show considerable variation, being the highest in elderly subjects.

The hereto pertinent literature data, however, represent a predominantly historical interest since modern therapy with antibiotics at its timely prescription should protect the sick from a fatal outcome.

RELATIONS OF IMMUNITY IN TYPHUS FEVER.

It is generally known that people who passed through TF, acquire a well marked, usually long-lasting immunity to reinfection. This refers also to laboratory animals, especially to monkeys and guinea pigs, which passed through an experimental TF infection.

The presence of acquired immunity in those who had had the disease is established authentically by clinical epidemiological observations. In laboratory animals which are subjected to inoculation with RPr the immunity is unquestionably proved by the failure of experiments at reinfection.

However, it should be stipulated that the stability of immunity in former TF patients is subject to individual variations. In some people it lasts practically throughout life, while in others, on the contrary, it gradually weakens with restoration of susceptibility to TF infection---therefore cases of second TF are observed in formerly recovered TF patients.

In men and animals who had TF infection, the acquired immunity to reinfection finds its expression also in the serum which shows the ability to prevent against inoculation with RPr, when introduced in advance or simultaneously to the susceptible animal (e.g., guinea pig).

Together with the anti-infectious properties, the serum of persons and animals who had TF infection shows the ability to neutralize the toxic substance of RPr, which can be easily demonstrated, for instance, at administering a mixture of a lethal dose of toxic substance together with a suitable amount of serum to mice.

The mentioned observations settle the concept about the two-faced immunity in TF---an antiinfectious and an antitoxic immunity, with clear representation of both mechanisms of non-susceptibility in the immune sera of former patients.

These observations on the antitoxic and antiinfective mechanism of immunity in TF are briefly discussed in detail in the following form.

ANTITOXIC IMMUNITY.

The RPr form a toxic substance, similar in its action to bacterial toxins, but not separable from the body of rickettsiae. At the same time, it is very labile, and it is detected only in live rickettsiae. White mice are particularly sensitive to the action of the toxic substance of RPr. These animals, at intraabdominal or better at intravenous introduction of a sufficient dose of live rickettsiae (Dlm) die during a day with marked disturbance in the cerebral blood circulation (A.P. AVTSYN).

By analogy with bacterial toxins, the toxic substance of RPr, both in natural infection and also in artificial immunization, cause the production and accumulation of a specific antitoxin in the organism. As a result, the serum of former patients or of the vaccinated acquires the ability to neutralize the toxic action of rickettsiae with a display of regularity peculiar to antitoxic sera in general. The antitoxic properties of TF sera are measured in units (AE), each of which neutralizes 2 Dlm of the toxic substance of rickettsiae for mice.

The antitoxic immunity which develop in TF is very easily revealed at the study of the neutralizing capacity of sera in TF patients and convalescents. It is expressed in titres of AE, determined in respect to Dlm of the toxic substance, as this was mentioned above.

Thus, according to the data of S.I. RATTNER, M.A. DOBZHIN, and M.M. ALEVSKII (1945), in TF patients the formation of antitoxin in the blood is shown already during the course of the disease, but during the ailment the serum titres are usually low (16-256 AE), and only at the end of fever are they slowly rising (sometimes up to 800 AE). However, the antitoxic titres reach their peak value in the convalescent period, especially on the 12th-15th day, sometimes reaching values from 2000-4000 to 8000-16000 AE. Further on, the titres gradually decrease, and after 3-4 months they are seen already within the range from 500-2000 AE, yet maintaining a level of low values even after years (16-64 AE).

Such is the picture of antitoxic immunity which develops under the effect and as a result of TF infection.

But such an order of antitoxic immunity is also reproduced in case of an artificial immunization, although it is here of much lower titres. Thus, according to the observations of N.O. BRONSTEIN (1945), 2-3 weeks after double and triple inoculations of killed vaccine prepared from RPr, the antitoxin titre in the blood of inoculated individuals reaches 130 AE, with a maximum of 250 - 500 AE, after which it quickly drops, expiring in 6 months.

Let us pass now to the characteristics of antiinfectious immunity.

#### ANTIINFECTIOUS IMMUNITY.

Antitoxic immunity, by providing the organism with resistance to an intoxication, does not provide it, however with an ability of direct response to the pathogenic agent of the infection. In other words, antiinfectious immunity in sensu proprio refers to phenomena sui generis, and provides other mechanisms which are different from the antitoxin neutralizing capacity of the organism, and is evidently related to phagocytosis, as this was established in regard to rickettsiae in our laboratory by I.N. KOKORIN (1957). The independence of antiinfectious immunity from antitoxic resistance to TF is illustrated particularly so that the TF pathogenic agent can be preserved in the organism in presence of a high concentration of antitoxin in the blood (M.M. ALEVSKII), while on the other hand, among people who are resistant to TF, the antitoxin titres in the blood can be very low (N.O. BRONSTEIN).

The phenomenon of antiinfective immunity, in addition to its clinical epidemiological detection in people who had had TF, is detected with the greatest detail in laboratory experiments on animals.

Thus, the antiinfectious immunity is rather clearly manifested in guinea pigs which had had TF infection, with subsequent inoculation of their cerebral TF virus under the control of simple thermometry. Under these conditions, the control pigs respond to the infection with cyclically running fever while the fever is completely absent in those guinea pigs which previously had had TF infection.

However, the antiinfectious immunity is still more clearly manifested with the aid the peritoneal test which was elaborated in our laboratory and was described above.

Let us recall that the peritoneal form of TF infection which is produced in male guinea pigs at intraabdominal introduction of a sufficient dose of Rickettsiae prowazeki, is characterized by the following signs.

Clinically this experimental rickettsiasis in guinea pigs appears with a febrile reaction which, starting from the 2nd to 3rd day after inocu-

lation, reaches its peak on the 4th to 5th day after inoculation, and then on the 5th to 6th day, it is reduced to a subfebrile temperature. Microbiologically these forms of experimental infection are characterized by simultaneous multiplication of rickettsiae in the mesothelium of the peritoneum and in the mesothelium of the testicular tunica vaginalis (periorchitis) with maximum accumulation of the microbes on the 4th to 5th day after inoculation of the guinea pigs, and with their subsequent disappearance on the 7th day.

In difference from the above, in immune guinea pigs at the arrangement of the peritoneal test, on the one hand there is no temperature reaction, and on the other hand, a rapid self-cleaning of the peritoneal and tunica vaginalis mesothelium from the rickettsiae is observed, which can be seen by the naked eye (Table 9 and 10).

The described cases illustrate the active acquisition of anti-infectious immunity. But the latter can be produced or revealed in analogous experiments on guinea pigs also in a passive form, i.e., with the aid of immune sera taken from guinea pigs or from people who passed through the disease. Even in these cases, the antiinfectious immunity is manifested very distinctly at intraabdominal introduction of the serum in sufficiently large dose (2 ml) simultaneously with a corresponding dose of RPr. Here, our experiments showed that in guinea pigs the protective action of the serum is fully manifested after passing through an experimental TF infection, and with the use of human serum it preserves its effectiveness in the peritoneal test even after years of a former TF.

As to the mechanism of antiinfectious immunity of the individual passively treated with the immune serum, it is probably related to the bactericide and opsoninic effect of the latter.

This is the general outline of the characteristics of antitoxic immunity in TF in the reflection of suitable laboratory tests.

#### FEBRILE REACTION IN GUINEA PIGS AT THE PERITONEAL TEST

Day	1	2	3	4	5	Rickettsiae
Control guinea pigs	-	39,8--39,5° 40,1--40,5°		40,1--40,6°	40,4°	+++
Immune guinea pigs	-	39,3--39,2° 39,1--39,2°		39--39,1°	39,2°	-

TABLE 9

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RICKETTSIAE IN THE PERITONEAL SYSTEM OF GUINEA PIGS

Time of autopsy	Control pigs		Immune pigs	
	Peritoneum	Tunica vaginalis of testis	Peritoneum	Tunica vaginalis of testis
24 hours	+/+	+/+	+	+
48 hours	+/+/	+/+/	+	+/+
72 hours	+/+/	+/+/	-	-

TABLE 10

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INFECTIOUS IMMUNITY IN TYPHUS FEVER.

Together with the antitoxic and antiinfective acquired immunity in TF, the presence of infectious immunity must be also considered, i.e., the forms of resistance related to the presence of the infection itself.

The French investigators G. BLANC and NOURI (1937), by using crossed inoculation of guinea pigs with the virus of rat TF and epidemic (louse-borne) TF, have established that inoculated guinea pigs are resistant to the transmitted superinfection not only during the period of manifested infection, but also in its incubation period.

In connection with the quoted observations in our laboratory independent researches were conducted on the problem of infectious immunity in TF in guinea pigs, including the attitude of inoculated pigs to superinfection in the incubation period. The search of resistance to superinfection was carried out in serial experiments. Here, the guinea pigs were inoculated with cerebral passage virus by the usual method, and the search for immunity was made by the above described peritoneal test with the employment of different doses of RPr at different phases of the infectious process, in the form of a suspension made from rickettsial lungs of intranasally inoculated mice. The summary results of this complicated experiment are presented in Figure 26.

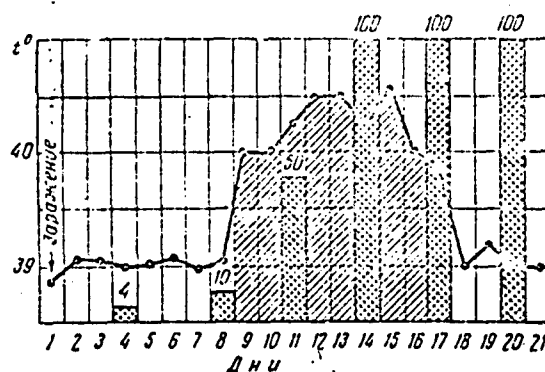


Figure 26. - Movement of immunity in experimental TF in guinea pigs. The columns correspond to the immunity level manifested by the number of infective doses of *Rickettsia prowazeki*.

As it can be seen from Figure 26, during TF infection, so far as it is reflected in the experiments on guinea pigs, a very characteristic symptom of resistance is shown in the inoculated organism to superinfection. This resistance is distinctly expressed already from the first day of incubation, and it obviously arises in the organism shortly after inoculation, and subsequently reaching a certain maximum, it remains at the level of the latter during the whole febrile period. In other words, in TF the whole infectious cycle---from the inoculation of the organism to the disappearance of ailment---is immunologically characterized as a state of "infectious" or "non-sterile" immunity.

After the disappearance of the pathogenic agent, the status of infectious immunity as an incomplete form of non-susceptibility is exchanged with a state of complete post-infectious immunity. However, in a number of cases, in agreement with the retention of the pathogenic agent in the organism, the immunity is preserved for a certain time, and in guinea pigs even the features of non-sterile immunity.

The above noted fact of revealing a resistance to superinfection due to inoculation already in the incubation period is of a special interest for the explanation of the rapid reproduction of immunity with the use of live TF vaccines. We shall return to this problem in the section on the specific prevention of TF.

#### THE PHENOMENA OF CROSSED IMMUNITY IN THE TYPHUS FEVER GROUP.

As it is well known, in addition to epidemic TF whose pathogenic agents are the RPr, to the typhus fever group of rickettsias, belong also the epidemic, or rat TF, caused by R. mooseri. The pathogenic agent of these related rickettsias have a common thermostable antigen, similar by the way to the antigen OX<sub>19</sub> (CASTANEDA and TSIA, 1933; CASTANEDA, 1935), at the same time being different by the presence of a specific thermolabile antigen (J. CRAIGIE et al, 1946). In correspondence with the indicated, the sera which are immune to one form of rickettsiae, in the experiments on animals will give full protection against a homologous infection, but at the same time to a certain degree they can protect also against heterologous inoculations (ZINSSER and CASTANEDA, 1932). The same is also observed at crossed neutralization of the toxic substance of both types of rickettsiae (CRAIGIE et al, 1946).

Due to the predominance of the homologous action of immune sera over the heterologous action, in the indicated experiments not only the relation of RPr, and R. mooseri was shown, but simultaneously their difference was also illustrated.

Compared with the quoted experiments of passive protection, the phenomena of crossed immunity in the described group of rickettsias appear at the collation of active post-infectious immunity.

Thus, numerous observations of various authors showed in coordinated forms that animals and people who passed through one type of rickettsias of the discussed group usually become non-susceptible to the other type.

As an illustration of this, we quote the findings of G. SPARROW (1935) on cross immunity in guinea pigs in endemic and epidemic TF.

Thus, according to his numerous experiments, guinea pigs which passed through an infection of epidemic TF or rat TF (R. mooseri), showed full immunity to inoculation with the pathogenic agent of epidemic typhus (RPr) in

86%, and under the reverse condition in 94%. In other words, crossed immunity in guinea pigs was manifested in the majority of cases.

It is very important here to mention that crossed immunity is well reproduced also after the transmission of an asymptomatic infection, as this was especially found in experiments by the peritoneal test in our laboratory, which is illustrated in Table 11 (E.M. GOLINEVICH).

In correspondence with the above data, French authors in their time were successful in North Africa to make extensive experimental active immunization of the population with live vaccine against epidemic TF, by producing asymptomatic forms of rat TF with the aid of an "attenuated" Mooser virus.

TABLE 11

No. of infect. doses	Inoculation with R. mooseri			No. of guinea pigs	Immunity to R. prowazeki	
	Mode of inoculation	Average incubation	Fever (in days)		full	partial
100	Intraabdom.	10 days	3.8	13	13	-
100	Subcutan.	Asymptomatic infect.		20	20	-

Together with this, it is not without interest to note that in difference from infection, even though it would be in an asymptomatic form, with the aid of killed vaccines, a cross immunity in the group of TF rickettsiases is very weak, especially at immunization of guinea pigs with killed vaccine made of R. mooseri against their infection with R. prowazeki. In the experiments of our laboratory, with searching the immunity by peritoneal test, a crossed protection was practically never successful at all, even under conditions of hyperimmunization with killed vaccine (E.M. GOLINEVICH).

SEROLOGY AND SERODIAGNOSIS OF TYPHUS FEVER

In people and in animals, TF infection causes the formation of specific antibodies to the antigen of RPr. Among these antibodies in the order of chronology of their discovery we have to name: agglutinins (OTTO & A. DIE-TRICH (1917); EPSTEIN, 1920); complement-binding antibodies (E. JACOBSTAL, 1917; G.V. EPSTEIN, 1922); opsonines (G.V. EPSTEIN, 1927); precipitins (C. LIM, and T. KUROTSCHKIN, 1929; R.D. ZAITSEVA, 1952); toxin neutralizing antibodies (GIROUD, 1936; GILDEWEISTER & HAAGEN, 1940); antibodies causing specific hemagglutination (S. CHANG, E. MURRAY, J. SNYDER, 1954; E.M. GOLINEVICH and collaborators, 1955-1960).

In agreement with this, various serodiagnostic reactions were worked out for TF infection: ---the reaction of agglutination of rickettsiae (RAR), the reaction of complement binding (CBR), the toxin neutralization reaction (TNR), the hemagglutination reaction (HAR). Most recently, the reaction of gel precipitation (Z.A. VORONOVA, 1963) was added to these.

However, all these reactions became accessible for practice only during the last 20 years, after the methods of mass accumulation of RPr were invented under laboratory conditions in the lungs of mice (DURAND & SPARROW, 1940), and in egg cultures (COX, 1940).

As it is well known, instead of the above mentioned serological reactions with an antigen made of RPr, starting from 1916, for a long period the serological diagnostics of TF was successfully made with the help of the WEIL-FELIX reaction, i.e., agglutination of an antigen from *Proteus* OX<sub>19</sub>. As the researches of CASTANEDA and ZIA showed in their times (1933), the specificity of the Weil-Felix reaction is based upon a thermostable antigen common in *Rickettsia prowazeki* and in *Proteus* OX<sub>19</sub>.

It should be mentioned that the use of the WEIL-FELIX reaction is fully justified for the serodiagnosis of epidemic (classical) TF, by being in the diagnostic titres (1:200 and more) already on the first week, and showing almost 100% positively in the second week of sickness.

However, in the different countries, it was shown in concord that the WEIL-FELIX reaction is not very suitable for the serodiagnosis of sporadic cases (forms of TF, in which it is negative in a larger percentage of the cases (e.g., up to 61.5% according to the data of Polish authors)---a symptom so marked that it is a characteristic of the features of sporadic TF (MURRAY et al, 1950; KOSTSHEVSKII & VOITSEKHOVSKII, 1959; COMBIESCU, ZARMA, et al, 1959; E.M. GOLINEVICH, and collaborators, 1962).

Finally, at the present time, the WEIL-FELIX reaction lost its diagnostic importance---instead of it, the above mentioned serodiagnostic reaction with specific antigen made from RPr came into wide practice. Here, for the latter category of reactions, their indices are also considerably wide spread---they are used not only for the serodiagnosis of TF ailments, i.e., at clinical tasks, but more and more they are utilized for the solution of different epidemiological problems. In correspondence with the above mentioned facts, we discuss first the general characteristic of the more important serological reactions for TF infection, and then we shall separately discuss their use at the clinic and in epidemiology.

GENERAL CHARACTERISTIC OF SEROLOGICAL REACTIONS WITH AN ANTIGEN  
MADE OF RICKETTSIA PROVAZIKI.

In a comparative characterization, E.M. GOLINEVICH presents the more important serological reactions of TF infection in the following form.

1. COMPLEMENT BINDING REACTION (CBR)

As E.M. GOLINEVICH emphasizes, the complement binding reaction (CBR) "by its clearness and objectivity of results can be considered as the main reaction for the diagnosis of TF infection". It is important here to keep in mind that CBR permits not only to reveal the active forms of TF infection, moreover independently from the clinical severity of the latter, but it also assures a retrospective detection of infection, suffered in the remote and even very remote past. The latter circumstance makes CBR indispensable for the solution of a number of problems of an epidemiological order, as this will be exposed in detail farther below.

A circumstance of not a small importance is also that the complement binding antibodies are relatively stable, and therefore the sera which are subjected to examination with CBR tolerate the transportation very well, and they can be sent away in a dried form on paper (V.A. YABLONSKAYA).

In the lyophilic dried state, at preservation in vacuum, the sera can keep their activity for CBR generally for an indefinitely long period.

Finally, CBR can be made both with a corpuscular, expensive antigen of RPr, and with a completely solved antigen of the same rickettsiae, a preparation incomparably cheaper and easier for the access to mass preparation (E.M. GOLINEVICH, 1952).

In agreement with the above indicated, CBR is accepted as the main serological test both in the USSR and in other countries, including Romania, Poland and the USA.

For the revelation of active forms of TF infection, in case of a single examination, CBR is diagnostically sure at a minimum titre in the order of 1:160 to 1:320, and for a retrospective diagnostic it begins with a titre of 1:10.

2. REACTION OF INDIRECT HEMAGGLUTINATION (HAR).

As it is well known, the basis of this highly sensitive test is the agglutination of erythrocytes (for instance, sheep red cells) by a specific serum, which erythrocytes have absorbed the antigenic component (haptene) of RPr. Being a specific test, in difference from CBR, HAR is obtained only during the active phase of TF infection, or in the early period of convalescence, and, thus, it is not suitable for a retrospective diagnosis of individual infections. At the same time, which is particularly important, HAR permits to differentiate fresh or relatively not old forms of TF infection from retrospectively diagnosed forms. As observations show, in the TF patients, HAR appears in the beginning of the second week of sickness, and, with some permanence and some strength, it can keep on roughly until 6 to 11 months after the end of sickness.

Just as CBR, HAR can be also well or strongly marked not only in the typical forms of TF, but also in all variants of obliterated, atypical and asymptomatic course of TF infection, as this was precisely shown in our

laboratory, especially in the reproduction of vaccinal forms of infections with the inoculation of the E strain of RPr (E.M. GOLINEVICH and V.A. YABLONSKAYA, 1962).

HAR is specific already in a serum dilution of 1:250, but the safe diagnostic titre for this test is equal to dilution 1:100. Not infrequently, including also the asymptomatic forms of infection, the reaction is found at very high dilutions (e.g., 1:12 800). HAR is found in the majority of cases of TF infection, but sometimes it can be absent when the CBR is positive.

Specially valuable results for both the clinic and for epidemiological examinations can be obtained by the combination of CBR and HAR.

### 3. THE AGGLUTINATION REACTION OF RICKETTSIAE (RAR).

The agglutination reaction, which was known in the past under the term of WEIGL reaction, is a very old serological test for TF. As E.M. GOLINEVICH points out, "to the advantages of RAR belong its simplicity and technical general accessibility. Its shortcomings are the not always well-marked clearness of results which does not exclude subjectivity in its evaluation. Moreover, RAR is made with corpuscular antigen prepared from RPr, i.e., a very expensive preparation, and very troublesome for mass preparation".

RAR is not suitable for an individual retrospective diagnosis of TF infection, since already a few months after the passed sickness the agglutinins disappear from the blood. In difference from the complement binding antibodies, the agglutinins to RPr are relatively unstable. Therefore, sera for agglutination should be examined in the fresh form. They are not suitable even for far transportation. Their dispatch in a dried form on paper is not permissible.

For the sake of economy with the antigen, various authors proposed various variants of microagglutination with the observation of results in drops or in preparations on a slide. All these modifications, however, are not suitable for wide practice, since they can lead to erroneous conclusions.

G.S. MOSING proposed a "drop" agglutination in small volume with the use of antigen in the form of a phenolized mixture of RPr made from the triturated stomachs of infected lice. As the investigations of Z.A. VORONOVA (1963) showed, which were made in our laboratory, the mentioned antigen of MOSING actually is a mixture of corpuscular antigen (bodies of rickettsiae) and dissolved antigen in the form of an autolysate of rickettsiae. In mixture with immune serum, the reaction with MOSING's preparation proceeds as a combination of precipitation and agglutination. Due to the presence of dissolved antigen in this preparation, the antigen may also give CBR in considerable dilutions (similar dilutions of corpuscular antigens are not effective).

The diagnostic titre for the RAR with the ordinary corpuscular antigen is equal to 1:160 dilution; the same is 1:40 with the use of MOSING's antigen with the drop setting of the reaction.

### 4. REACTION OF NEUTRALIZATION OF THE RICKETTSIAL TOXIN.

The reaction of toxin neutralization, according to the findings of American authors, (MURRAY, SNYDER, et al., 1952), is one of the most sensitive tests for retrospective detection of TF infection. This reaction is set up on mice with the use of a certain amount of mixture of live

Rickettsiae prowazeki as the toxin. This toxin is neutralized with the addition of appropriate dilutions of immune serum. The results are estimated by the percentage survival of test mice in presence of lethal intoxication of control animals.

In view of the complexity of the setup, the described test is used only in suitable laboratories for the solution of special problems.

In conclusion, let us point out still the skin allergy test, which was last year (1962) propagated in the laboratory of K.N. TOKAREVICH (1962). For the setup of this test, they used a specially titrated lysate of RPr, which is injected intracutaneously in the amount of 0.1 ml on the flexor surface of the forearm. The reaction is read after 24 and 48 hours, and if the outcome is positive, it shows in the form of a circumscribed redness and a slight infiltration at the site of inoculation. As the observation of K.N. TOKAREVICH and his co-workers showed (D.A. GVOZDILOVA, 1961), an allergic reaction is obtained regularly both in case of an active TF infection, and also in people who had had it in the past, and it shows a good correlation with CBR. The cutaneous allergy test is especially indicated for retrospective detection of TF infection in case of mass examination of the population where the use of CBR is inaccessible.

Such are the general characteristics of sero-immunological reactions used for the exposure of TF infection in its active phase and retrospectively.

Let us go over now to the use of the indicated reactions in a clinical setup and in case of epidemiological investigations.

#### SERODIAGNOSIS OF TYPHUS FEVER AT THE CLINIC.

Summarizing the findings in the literature and the experience in our laboratory, in his manual on the serodiagnosis of TF, E.M. COLENEVICH shows that roughly in about 80% of TF patients specific antibodies are found in their blood with the aid of one or another reaction (CBR, HAR, RAR), beginning from the 5th-7th day of sickness, and on the 10th day the antibodies are found in almost all patients. The appearance of agglutinins (RAR) can be outstripped in a number of cases by the appearance of complement binding antibodies (CBR). Here, it is characteristic that in patients with a second infection of TF positive CBR is found usually in a very early period in comparison with patients with a first infection. We should specially mention the regular finding of distinctly or strongly marked HAR with titres within the ranges of 1:1000 to 1:64 000 during the 7th to 10th day of sickness.

After the 5th to 7th day of sickness, the titres of antibodies begin to increase, and, having reached the maximum by the 15th day from the start of the disease, they will then gradually decrease. Here, the titres of CBR and RAR are characterized by relatively moderate elevation and decrease, while for HAR very high titre values are characteristic at the height of their increase (up to 1:64 000), and their drop is relatively rapid. The prescription of antibiotics can reduce the formation of antibodies, especially if they are prescribed early.

TABLE 12

Serological reaction	Day of Sickness		5-7	8-10	11-15	16-20	21-25	26-70
Agglutination reaction(RAR)	% of positive reaction		84,8	85,5	96,5	100	100	100
	Range of titres		40-1280	80-2560	80-2660	80-2560	80-2560	-
Complement binding reaction(CBR)	% of positive reaction		63,6	97	100	100	100	100
	Range of titres		40-2560	40-2560	80-5120	320-5220	80-2560	-



Table 12 gives the RAR and CBR during the TF sickness and in the immediate convalescence (according to the data of E.M. GOLINEVICH and V.A. YABLONSKAYA, 1953).

As to all three reactions (RAR, CBR, HAR), the same time relations of their appearance and the titre ranges are shown in Table 13 according to the data of E.M. GOLINEVICH.

TABLE 13

Day from the start of the disease			
	RAR	CBR	HAR
5.-7	1:80--1:320	1:80--1:640	1:150--1:800
10.-15	1:160--1:2560	1:160--1:10240	1:1000--1:64 000
20.-30	1:160--1:1280	1:160--1:5120	1:1000--1:16 000

The movement of increase and decrease of the serological reactions, as given in Tables 12 and 13, during the disease and in the convalescent period is very characteristic for the serology of TF infection. In agreement with this, the serodiagnosis of TF is also the most reliable with repeated examination of the blood at intervals of a few days with consideration of the increase or drop in the titres depending upon the phase of infection. Here, in those cases where a non-specific activation of CBR can be suspected in the order of its anamnestic resuscitation, a parallel setup of HAR is especially important which, as we have already seen, reveals the active state of TF infection.

As to the time of preservation of the specific serological reactions in people who had had TF infection, the hereto pertinent findings are presented in the following form (E.M. GOLINEVICH): the agglutinins to RPr (RAR) disappear in convalescents about 8-10 months later. The hemagglutinins (HAR) disappear in 1-3-6 months. The complement binding antibodies (CBR) usually are preserved for years and decades, and upon this is based also the retrospective serodiagnosis of TF infection by CBR, which makes possible to reconstruct the history of TF infection of the population.

As a supplement to the above exposed facts, we have to quote a number of findings for the characteristic of the value of serodiagnosis in the differentiation of the residual forms, resp. of sporadic TF, which frequently runs in a mild form, and makes its clinical diagnosis difficult.

Thus, according to earlier published collected data for the Russian Soviet Federative Socialist Republic, among 832 studied patients who had sporadic TF and were hospitalized in several hospitals for some years, in 75, i.e. in 9.1% the diagnosis was established only on the basis of serological examinations by CBR, at which the percentage in the various hospitals varied from 4.5 to 15% (P.F. ZDRONOVSKII, 1960).

The quoted data on the large percentage of TF sickness cases of the sporadic type which were diagnosed only serologically by CBR and HAR, are not exceptions. They completely agree with similar observations of various authors in other countries where they experienced similar stages of the residual forms of TF.

Thus, in Romania where during 1952-1962 TF was systematically studied under the leadership of Prof. COMBIESCU, and after his death (1962) under the guidance of Prof. ZARNEA, in the period of the greatest spread of epidemic TF (1953-1955), at the diagnosis of the disease only 10% of the cases could be found by CBR alone.

In the following years, on the basis of an energetic control of pediculosis and the subsequent elimination of the epidemic forms of TF and with the ultimate preservation of only the sporadic forms of the disease (1961-1962), the detection of TF ailments in 48% was achieved only due to the serological diagnosis by CBR (from the report of Dr. J. BUZDUGAN, 1963, according to the communication of E.M. COLINEVICH).

In agreement with the above, the detection of mild forms of TF infection of the sporadic type does not seem to be a rarity even among polyclinical patients, especially with the practice of preventive hospitalization of febrile ambulatory patients with different diagnoses (K.N. TOKAREVICH et al, 1958).

SEROLOGICAL RESEARCH IN EPIDEMIOLOGICAL EXAMINATIONS OF  
TYPHUS FEVER AND THE PROBLEM OF SPECIFICITY OF CBR IN  
LOW TITRES

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Together with its huge clinical importance, the serodiagnoses of TF infection also has a great epidemiological significance. Thus, retrospectively revealing TF sickness in the past, and thus reconstructing its history, in mass examinations of the more aged section the serodiagnosis with CBR makes possible to determine the immunological structure of the population in regard to TF with simultaneous exposure of the presence or absence of an active source of infection at the time of examination. On the other hand, by revealing all forms of TF infection independently from the clinical manifestation, including also cases of subclinically running infection, and differentiating the past contaminations and ailments from the current ones, the combined serodiagnosis with CBR and HAR is indispensable for the most accurate study of the foci of TF and their origin.

In general, it can be asserted with complete authenticity that the epidemiology of TF cannot be valuable at the present time if it does not use seroimmunological methods of examinations.

For the problem of epidemiology, as it is evident from the above facts, mass examination of the population of the studied locality by CBR acquires a very important value for the retrospective exposure of its affliction with TF infection in the past, which at the same time also defines the immunological structure of the population in regard to TF. In connection with this, two questions of principal importance arise:

- 1) for what times in the past does CBR retrospectively reveal the TF infection, and
- 2) what titres of CBR are acceptable for the retrospective detection of TF infection at similar examinations.

Let us answer first of all the first question.

As it was already shown in the general characteristic of TF serological reactions, CBR is maintained for years and for decades after a passage through TF infection. This situation is clearly illustrated in Table 14, which presents the summary of the hereto pertinent observations of E.F. EPSTEIN from the laboratory of K.N. TOKAREVICH.

TABLE 14

Time after sickness in years	Number of examinees	Results by CBR		
		Negative	Positive	
			Absol. number	%
$\frac{1}{2}$ - $1\frac{1}{2}$	20	-	20	100
2 - 10	54	10	44	81
11 - 31	155	46	109	70
TOTAL	229	56	173	75

These are the chances of detecting TF infection, according to the data of E.F. EPSTEIN (1952), within the time limits from  $\frac{1}{2}$  -  $1\frac{1}{2}$  year to 31 years. It should be mentioned that such a detection can be made even at a much later time both according to the data in the literature and according to the experience of our laboratory.

Thus, for instance, H. PLOTZ and co-workers observed positive CBR in people who had TF 26 and 43 years before.

In the author of the book, who had had TF in 1920, on January 1963, i.e., also after 43 years, the CBR was positive in a dilution of 1:40.

We see thus that at retrospective examinations, in the majority of cases the CBR is manifested in titres from 1:5 - 1:10 to 1:20 - 1:40, with an absolute predominance of the titres from 1:10 to 1:20. To what extent are these low and relatively low titres specific for a retrospective detection of TF infection?

TABLE 15

Number of Examinees	Among them positive by CBR		Positive titres of CBR (in %)				
	Abs. number	%	1:5	1:10	1:20	1:40	1:80-1:160
3257	330	11	18	46	26,4	7,3	2,3

In the foreign literature, the question about the specificity of CBR in titres which are observed at the retrospective detection of TF infection, is in the following form.

A large group of American researchers, with PRICE (1958) at their head, examined 1708 persons during 5 years with CBR using an antigen made from RPr. These persons have emigrated to the USA not less than 25 years ago from Eastern and Southeastern Europe. Almost in 30% of these people, the researchers found positive CBR, moreover, in the titre of 1:5 in 20%, 1:10 to 1:20 in 63%, and higher than 1:20 in 17%. The sera of 1:5 and 1:10 titres were

additionally examined by the neutralization reaction with rickettsial toxin. The latter showed a positive reaction in all cases, which confirmed the specificity of the CBR in the indicated dilutions of 1:5 and 1:10. Moreover, it was shown that out of 100 sera which were negative with CBR, a positive neutralization reaction could be obtained in 5, which proved the great sensitivity of the latter compared with CBR (see below).

Finally, 308 persons with positive CBR were examined repeatedly not less than three times in a year, and in 8 out of them during this period the positive CBR and the reaction of toxin neutralization changed to a negative one.

As a control, the American authors examined 481 sera of people who were born in the USA, and by age corresponded to the group of the serologically studied immigrants. In all examinees the CBR was negative. With supplemental examination of the sera of 120 persons, negative results were also obtained by the toxin neutralization reaction.

Finally, the sera of 201 persons were examined with the CBR who were born in the USA from parents with positive reactions and who were living together with them. Not a single one of them gave a positive reaction in any serum dilution used. It should be mentioned that the revealed observations of PRICE and his co-workers, published in 1956, were fully corroborated by the collected observations of MURRAY, SNYDER et al., which refer to 1952. The latter authors observed positive results in 20 out of 50 immigrants with their complete absence in a control group of people born in the USA and Canada. Here the mentioned authors established that for a retrospective exposure of TF infection the most sensitive test is a serological response to the introduction of a killed vaccine of RPr, i.e., the effect of revaccination. Close in sensitivity to this test is the reaction of neutralization of rickettsial toxin, and, finally, the third place belongs to CBR.

Numerous examinations in our laboratory and the findings of other domestic authors, in correspondence with the above exposed facts and observations of American investigators, fully confirmed the unquestionable specificity of CBR in titres which interest us and which are shown at retrospective examinations of the population for TF infection.

Thus, according to the data of G.F. DOLGOV & N.M. BALAEVA, a good correspondence was shown between CBR in titres 1:10 - 1:40 and the neutralization reaction of rickettsial toxin (Table 16).

The specificity of CBR is also very convincingly demonstrated by the results of mass examination with this reaction of children and youth of the collectives of different cities of the RSFSR and the Belorussian Soviet Socialist Republic, located in the zone of sporadic TF, i.e., in absence of active sources of infection (Table 17).

Thus, at the examination of 1009 children and young people only in 1 case, i.e., in 0.1% of the examinees was a positive CBR observed in a titre of 1:10. At the same time, at a parallel examination of 2430 inhabitants of the same zone in the 30 to 60 years age group, a positive CBR of the titre of 1:10 and higher was retrospectively detected in the average limits of 11.7 to 17%.

Таблица 16

Титры сывороток по РСК	Число исследованных сывороток	Результаты исследования по реакции нейтрализации	
		положительные	отрицательные
1:40	6	6	0
1:20	19	19	0
1:10	31	31	0
ниже 1:10	34	22	12
Сыворотки отрицательные по РСК от перенесших сыпной тиф в прошлом	10	1	9
Сумма	100	79	21

TABLE 16

1. Titre of sera by CBR; 2. Number of examined sera; 3. results of examination by the neutralization reaction; 4. positive; 5. negative; 6. less than 1:10; 7. Negative CBR sera of persons who had had typhus fever in the past; 8. Sum.

Таблица 17

Авторы	Число обследованных детей и подростков	Возраст в годах	Положительная РСК (1:10)	Отрицательная РСК при разведении 1:10
Г. Ф. Долгов,	322	2 месяца 6 лет	0	322
К. Н. Токаревич, Кан-Шу-тин.	200	3-12	0	200
Г. Р. Газизова,	107	7-12	0	107
В. А. Яблонская и др.	380	7-12	1	379
Всего . . . . .	1009		1	1008

TABLE 17

1. Authors; 2. Number of examined children and youth; 3. age group in years; 4. Positive CBR (1:10); 5. Negative CBR in the 1:10 dilution; 6. 2 months to 6 years; 7. G.F. DOLGOV; 8. K.M. TOKAREVICH, KANSHU-TSIN; 9. G.R. GAZIZOVA; 10. V.A. YABLONSKAYA et al; 11. Total.

In a group of 322 children in the age up to 6 years, especially thoroughly studied by G.F. DOLGOV, the results of CBR examinations are given in detail in the following form (Table 18).

Таблица 18

Возраст	Месяцы	Годы					
	2-11	1	2	3	4	5	6
Процент исследованных сывороток по возрастам	6	10	12	13	20	19	20
Результаты по РСК	Отрицательные						

TABLE 18

1. Age group; 2. months; 3. years; 4. percentage of the examined sera by the age groups; 5. results by CBR; 6. Negative.

For the characterization of the sensitiveness and reliability of CBR it is not without interest to introduce an observation of G.F. DOLGOV who in a two-months child found a positive CBR with a titre of 1:10. It was shown, however, that his mother had had TF and had a positive CBR in the titre of 1:160. In other words, in this case there was transfer of the specific antibodies from the mother to the fetus.

The following experiment, conducted by G.F. DOLGOV in the Byelorussian Soviet Socialist Republic, in its turn convincingly illustrates the specificity of CBR with rickettsial antigen. On the territory of the Byelorussian Soviet Socialist Republic, in the period of occupation of the area by Fascist troops during the Second World War, a large TF outbreak occurred which is retrospectively reflected in positive CBR with an antigen prepared from RPr. But on the territory of the Byelorussian Soviet Socialist Republic tick-borne rickettsiasis is absent, and the Q-fever is not known. G.F. DOLGOV made serological tests on 729 persons in one of the BSSR (Byelorussian) rayons with a parallel setup of CBR with three antigens:---one from RPr, one from tickborne typhus, and one from rickettsias of the Q-fever. The results of this complex examination in age groups are shown in Figure 27.

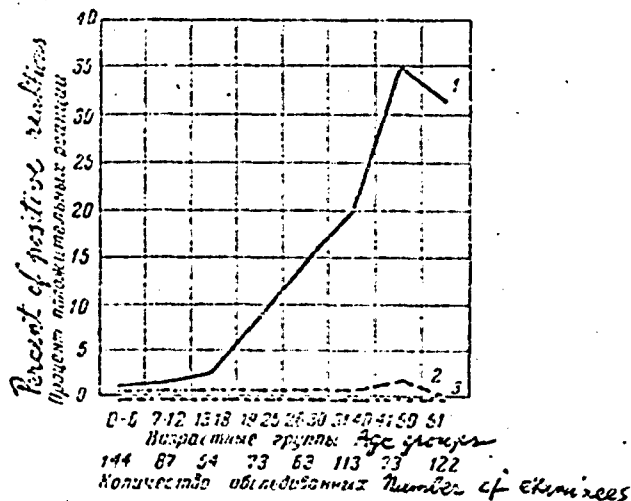


Figure 27. - Graphs of distribution of positive CBR by age groups made with antigens from *R. prowazeki* (1), *R. burneti* (2), and of tickborne rickettsiasis (3) according to G.F. DOLGOV.

We can see thus that the curve of the retrospective detection of people, who were infected with TF, with the aid of CBR gave a typical picture of a rise with a peak characteristic for elderly groups. At the same time, with the use of an antigen from rickettsiae of tickborne typhus, CBR gave negative results in all examined persons, and with an antigen made from Burnet's rickettsiae, CBR gave a positive result only in a single case with a titre of 1:10 in the 41-50 age group.

The quoted experiment thus clearly shows that the observed curve of positive CBR with RPr antigen, with its progressive increase from the younger age group toward the older and elderly groups, cannot be considered just the result of a "serological maturation", as it could be suggested, considering such kind of hypothesis as was announced by a few authors in regard to the formation of antibodies (H. HIRSCHFELD, 1930).

Finally, the specificity of CBR with RPr antigen is excellently illustrated by the very close coincidence of its results with the results obtained in parallel examinations by the skin allergic test with intracutaneous injection of a lysate made from RPr. As it was established by DVOZDILOVA in the laboratory of K.M. TOKAREVICH, the indicated coincidence refers also to cases of TF sickness which were examined under clinical conditions, as well as to the results of retrospective detection of TF infection among the population which were subjected to TF outbreaks. The curve of positive CBR findings and positive allergic tests by age groups practically fully coincides in the latter case. As an illustration we quote one of the experiments of retrospective detection of TF infection by both reactions which are quoted by D.A. GVOZDILOVA:----among 174 examined persons positive results by CBR and by allergic tests were obtained in 54 subjects with full agreement of both reactions.

In correspondence with all this, it is completely clear that CBR with an antigen made from RPr, being completely a specific test, beginning with a serum dilution of 1:10, invariably gives a negative result in other infectious and somatic ailments, or it is shown in them at such a percentage which corresponds to the retrospective detection of TF infection in the past.

This situation is clearly illustrated in the summary Table 19, compiled according to the data of G.R. GAZIZOVA.

Диагноз	Количество больных	Количество сыворо- ток	РСК					
			отрица- тельная	1:5	1:10	1:20	1:40	1:80
а Клеточной энцефалит	22	22	20	2	—	—	—	—
б Переболевшие клеще- вым энцефалитом в 1954—1959 гг.	30	30	24	6	—	—	—	—
в Лихорадка Ку	30	57	57	—	—	—	—	—
д Переболевшие из оча- га лихорадки Ку	14	14	13	—	—	—	1	—
е Геморрагическая лихо- радка	37	49	48	—	1	—	—	—
ф Гripp (эпидемия 1959 г.)	237	394	385	4	1	1	4	1
г Туберкулез	7	14	14	—	—	—	—	—
з Брюшной тиф, паратифы	14	17	17	—	—	—	—	—
и Всего . . .	391	597	576	12	2	1	5	1

TABLE 19

1...Diagnosis; 2...number of patients; 3...number of sera; 4...CBR; 5...negative; а...Tickborne encephalitis; б...Persons who had tickborne encephalitis in 1954-1959; в...Q-fever; д...Persons who had had sickness from a Q-fever focus; е...Hemorrhagic fever; ф...Influenza (1959 epidemic); г...Tuberculosis; з...Abdominal typhoid, paratyphoid; и...Total.

Similar results were also obtained by G.F. DOLGOV who examined 1582 persons with CBR in one of the BSSR oblasts, afflicted at the time of examination by various ailments of non-TF etiology. The hereto pertinent results of the examination are summarized in Table 20.

a Клинический диагноз	b Число сывороток, исследованных по РСК	c Положительные результаты по РСК	
		1 : 10-1 : 40	d 1 : 80 и выше
1 Грппи	260	38	5
2 Острый катар верхних дыхательных путей	537	60	5
3 Прочие и простудные заболевания	296	38	0
4 Ревматизм, ангины	67	2	2
5 Пневмония, бронхит	66	5	2
6 Брюшной тиф, паратифы	66	12	0
7 Болезнь Боткина	58	3	0
8 Прочие инфекционные заболевания	90	26	3
9 Сердечно-сосудистые заболевания	42	7	1
10 Всего . . . . .	1582	191 (12.1%)	18 (1.1%)
		209 (13.2%)	

TABLE 20

a...Clinical diagnosis; b...number of sera, examined by CBR;  
c...positive results by CBR; d...1:80 or higher; 1...influenza;  
2...acute catarrh of the upper respiratory pathways; 3...other  
and common cold ailments; 4...rheumatism, angina; 5...pneumonia,  
bronchitis; 6...abdominal typhoid, paratyphoid; 7...Botkin's  
disease; 8...other infectious diseases; 9...cardiovascular dis-  
eases; 10...Total.

As it can be seen from Table 20, among the serologically examined 1582 persons with various clinical diagnoses, G.F. DOLGOV found positive CBR with RPr antigen in a total of 209 persons, or in 13.2%. These results fully coincide with the examination of 2770 healthy persons by the same CBR and at the same place in whom a positive CBR with titres from 1:10 to 1:80 was found even in 13%. In other words, among patients of non-TF etiology the positive CBR was manifested as a retrospective test for TF infection in the past, moreover, within the ranges of its findings in the population of the given locality.

At the analysis of positive results G.F. DOLGOV specially separated 18 cases of positive CBR with titres of 1:80 or higher. In case of supplemental examination of these patients with HAR, which differentiates active TF infection, in all cases he found negative results. Hence, the author made the reliable conclusion that in the mentioned group of 18 persons, evidently a revival of anamnestic CBR took place under the influence of an associated infection--a phenomenon known for a long time in respect to anamnestic reactions generally. Further on, G.F. DOLGOV specially singles out 2 cases of positive CBR in titres of 1:10 and 1:80 which he observed in two children of 3 years of age, without a TF anamnesis. At the moment of the examination the children suffered from rheumatism and pyelocystitis. The author considers these two findings as non-specific, constituting only about 0.1% in the examined group.

Thus, we see that the specificity of CBR with RPr antigen is demonstratively confirmed also in the mass control use of the reaction in patients with the most diverse ailments of non-TF etiology. As a retrospective test for TF infection in the past this reaction is displayed in patients within the range of its spread among the local population. As to the possibility of a non-specific CBR with rickettsial antigen, it is limited to very rare exceptions (2 doubtful cases in more than 2000 observations), which were observed within the ranges of reliability of the serological tests in general.



In conclusion, we introduce an illustration of the epidemiological agreement of the data of serological examination of a healthy population with CBR by age groups for localities which are in favorable and unfavorable conditions in regard to epidemic TF.

а Возраст (в годах)	7-12	13-15	19-25	26-30	31-40	41-50	51-60	Сум- марно
1 Число обследованных	380	361	1017	903	1156	837	437	5124
2 Положительная РСК, %	0,2	2,0	5,0	9,5	11,7	17,0	15,1	10
3 В среднем . . . . .								

TABLE 21

a...age (in years); b...Total; 1...Number of examinees;  
2...positive CBR, %; 3...Average.

In Table 21 the summary data are presented of CBR examinations of 5124 healthy people of different ages from 5 cities of the RSFSR which in the time of examination were free of epidemic TF. However, during the Second World War these cities were subjected to TF infection, but now have only individual sickness cases of sporadic typhus.

From Table 21 it is thus obvious that the smallest percentage of positive CBR concerns the age groups of 7-12 years and 19-25 years which are highly susceptible to TF. This proves that in the post-war years in the examined collectives the circulation of the virus was absent, or it was weak in difference from previous years (the war years) when it was extensive and caused a high percentage of positive reactions (11.7% - 17%) in people of the mature and elderly age groups. This picture of agewise distribution of positive CBR at the same time shows the absence of active sources of TF infection, which in its turn is illustrated by individual cases of sporadic typhus fever essentially among people of the mature and older age groups.

1 Возраст (в годах) обследованных	2 Земледельческий район I		3 Земледельческий район II	
	4 абсолютные числа об- следованных	5 % положи- тельных по РСК	6 абсолютные числа об- следованных	7 % положи- тельных по РСК
0-4	158	8	58	5
5-9	535	19	272	19
10-14	450	18	368	30
15-19	373	45	124	61
20-29	521	50	153	71
30-39	321	76	85	80
40-49	156	81	92	83
50 и >	158	80	112	77
8 Всего .	2 682	46	1 264	46

TABLE 22

1...Age (in years of the examinees; 2...Agricultural district I;  
3...Agricultural district II; 4...absolute number of examinees;  
5...% of positive cases by CBR; 6...absolute number of examinees;  
7...percentage of positive cases by CBR; 8...Total; 9...50 and  
higher.

A complete different picture of the level and age distribution of positive CBR with RPr antigen is noticed at serological examination of the louse-infested population of localities which are endemic for epidemic TF in presence of active sources of infection. This situation is clearly illustrated in Table 22 which summarizes the results of CBR serological examination of one of the rural districts of Peru where epidemic TF is endemic (according to the data of J. MONTROYA, M. JORDAN, J. FOX, et al., 1955).

Thus, we can see from Table 22 that for the localities afflicted with epidemic TF, in addition to the high average indices of positive serological findings (46%), very characteristic is the presence of large, high, and very high serological indices in the groups of infants and young folks of the population, which is in strong contrast with similar indices of the retrospective serological analysis of the population with residual forms of sporadic sickness cases (see Table 21).

Summarizing all the above made statements, we must make the unique conclusion as to the unconditional, undoubted specificity of CBR with RPr antigen in titres in the range of 1:10 - 1:20 and higher, employed at epidemiological investigations in connection with TF infection. In its turn this statement is the foundation of two inferences of fundamental importance:

- 1) the unquestionable reliability of the laws manifested by the serological methods of examinations, and
- 2) the unconditional necessity of employing these serological methods for a thorough epidemiological study of TF infection. As we have already remarked above, modern epidemiology of TF cannot be of full value without the use of serological methods of investigations and examinations of the population.

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EPIDEMIOLOGY OF TYPHUS FEVER - MEASURES FOR ITS  
CONTROL AND GENERAL PREVENTION

Two basic peculiarities characterize the modern study of TF epidemiology:---the first of them refers to individual analysis of epidemic and sporadic recurrent forms of TF; while the other is related to the wide use of sero-immunological methods for the detection of TF infection, as we have seen it already before, which in many respects were newly appropriated in recent years.

In correspondence with this, further on we expose separately the modern data on epidemic and sporadic TF, in the International WHO Nomenclature of Diseases, the latter being identical with Brill's disease, or more precisely Brill-Zinsser's disease. Such a subdivision of TF is not made to oppose its two forms, since epidemic and sporadic TF have one and the same pathogenic agents, and under suitable conditions (pediculosis) sporadic TF can pass over into epidemic TF. However, the clinical epidemiological characteristics of the two forms of TF are such that in a number of cases their undifferentiated description under the common term "typhus fever" could give a distorted presentation of the actual condition in a given locality or State in regard to TF infection. As an illustration of the above, it suffices to say that, e.g., according to the requirements of the International Nomenclature of Diseases, epidemic TF is subject to obligatory recording in that system, whereas sporadic typhus fever is not counted at all.

In the Chapter on the serology and serodiagnosis of TF, we already discussed the importance of sero-immunological examinations for the epidemiology of TF, without which modern epidemiology could not be full-fledged. Further on in the discussion, we will again return to this question, by illustrating the hereto pertinent epidemiological laws with data which accumulated in our country and abroad on the detection of various varieties of atypical and subclinical forms of TF infection, on the status and importance of the immunological structure of a population, and so on.

In the exposition of TF epidemiology, let us deal first of all with the modern characteristic of its epidemic, i.e., classical forms.

EPIDEMIC TYPHUS FEVER.

The epidemiology of epidemic, classical, or louse-borne TF, in correspondence with modern data, is based upon the following generally accepted theses:---a sick person is the only source of TF infection which is spread among the healthy susceptible subjects by means of body lice, and partly also, probably by head lice. The infected louse excretes the pathogenic agent of TF with its fecalia, by which the infection of people also occurs; moreover in infected lice there is no transovarian infection, wherefore they cannot be reservoirs of the TF virus, as this, e.g., takes place in a number of other rickettsias whose carriers are ixodide ticks. In the final result, the circulation of the TF virus is limited to virus rotation in the chain man-louse-man in a strict monoxenousness of the carrier.

At blood sucking on patients the body louse can become infected with TF virus at all phases of its metamorphosis after its hatching from nits, but the infectiousness of lice varies depending upon the time of the disease and its severity. Thus, according to the data of V.I. MITROFANOVA (1945), in the first week of the disease the infectiousness of lice is 42%, in the second week 34% and in the third week 26%; moreover, according to the data of G.S. MOSING (1937), in severe TF cases after a single blood sucking in the first week of the disease the infectiousness of lice reached 60-80%, and in the mild forms only 2-3%. In 5-14% the blood can be infectious for lice also in the incubation period 1-2 days before the start of fever in the patient (V.I. MITROFANOVA, 1945), and sometimes 1-2 days after the start of the fever

(positive findings in 3 cases out of 10 examinees---B.I. RAIKHER, 1939). According to the observations of G.S. MOSING, 1937), in addition to the time and severity of the disease, the infectiousness of lice is also influenced by the duration and the repetition of their blood sucking. Here, it should be considered that the body louse, which is living on linen, several times daily passes over to the body surface for blood sucking, with a length of the digestive phase of about 5 hours.

In correspondence with the above statements, as L.V. GROMASHEVSKII indicates (1947), "a TF patient is apt to serve as a source of infection during a period of not more than 20-21 days (the last 1-2 days of incubation, the 16-17 days of the febrile period and 1-2 days of apyrexia)".

Depending upon the infecting dose and the environmental temperature, the period of the presence of rickettsiae in the organism of lice can vary from 3 to 18 days. Under optimum conditions of infection, at a temperature of 30°-32°, rickettsiae are experimentally found from the 4th to 5th day of infection, and they reach the maximum multiplication in the gastric epithelium of lice on the 8th to 9th day (A.V. PSHENICHNOV, B.I. RAIKHER). In correspondence with the indicated, according to the data of B.I. RAIKHER (1944), the infectiveness of lice picked from a patient on the second day of his sickness was equal to 0; in lice picked on the third day was 1.49%; on the 4th day....3.55%; on the 5th day....3.68%; on the 6th day....5.4%; on the 7th day....5.9%; and on the 8th to 10th day...32.2%.

Under the effect of an infected blood sucking a specific rickettsiasis develops in lice with abundant multiplication of RPr in the epithelium of the stomach. This leads to destruction of the epithelium with the release of a mass of rickettsiae into the lumen of the gastro-intestinal tract, and their subsequent elimination with the fecalia. In comparison with an equivalent weight of infected lice, the contents of rickettsiae in the fecalia is considerably less, but due to their high concentration in the stomach, and in view of the high susceptibility of people to TF infection, the fecal excretions possess a sufficiently high virulence for man.

Human infection itself does not take place through the bite of infected lice, as this was originally proposed by CH. NICOLLE (1909), since virus is not contained in the salivary glands, and at the blood sucking, it is not excreted through the oral apparatus of TF lice (DA ROCHA-LIMA-WEIL, BREINL), with the exception of cases of a mechanical contamination of the proboscide apparatus with Rickettsiae prowazeki. According to the generally accepted opinion, based upon experimental and epidemiological observations, human infection with TF occurs through the fecal excrement of lice at their dropping upon the organism through superficial injuries of the skin which are formed at scratching; moreover, by the same mechanism, infection is possible also by crushing the lice. It is very important to remark that (according to the data of E.N. PAVLOVSKII and A. STEIN) the very act of the blood sucking of lice, connected with injection of saliva, promotes infection through the superficial injury of the skin. The matter is so that, according to the experiences of the mentioned authors, the saliva of lice contains the secretion of pod-shaped glands which has a local effect upon the human skin, being manifested in the form of papulae with the sensation of pruritus and burning, destruction of the epidermis, destruction of the vessels and superficial blood extravasations. Due to the inflammatory dilatation of vessels, the blood sucking of lice is facilitated, but at the same time the above mentioned lesions of the skin, arising at the site of the lousebite, assist in its excoriation, and thus prepare the "portal" for the penetration of the agents of TF infection which are contained in the fecalia excreted by infected lice.

In addition to the excoriation and small wounds of the skin, the possibility is also unquestionable that a TF inoculation occurs with the drop of fecalia and material from crushed infected lice upon the mucous membranes, especially upon the mucosa of the conjunctival sac of the eye or of the upper respiratory pathways. This has been known already long ago from corresponding experiments of previous authors (H. SPARROW, LUMBROSO, 1929); LEPINE, 1932; CH. NICOLLE and collaborators, 1935), and not so long ago, it was again confirmed in our laboratory with the application of fecal virus from infected lice upon the conjunctiva of the eye of cotton rats (G.M. DUTOVA, 1963).

The intralaboratory TF inoculation of the staff which works in presence of open sources of infection with formation of aerosols, undoubtedly happens by the aerogenous pathway, evidently with regard to the very high sensitiveness of the respiratory pathways to TF infection. Hence, the infection of the laboratory personnel which works with TF pulmonary rickettsias in rodents, especially in mice is practically almost unavoidable. Since at the same time, as we have seen earlier, the rickettsiae which are contained in the fecalia of lice can be preserved in the outside environment for a long time, sometimes for months, some authors emphasize the importance of dust infection in TF. Not overestimating the importance of such an infection, especially under laboratory conditions, the latter cannot be considered evidently when an infection is probable, for instance, through the clothes of a TF patient.

The human susceptibility to TF infection is very high. In particular, according to the data of A.V. PSHENICHNOV, TF can be provoked in man even by 1/5000 of louse larva infected with RPr. However, we have to distinguish on the one hand an almost general susceptibility of people to TF infection, and at the same time an individual variable capacity to classical TF sickness under the influence of infection with RPr. This position is especially clearly manifested at the study of the sero-immunological and clinical reactivity of the collectives to inoculations with the attenuated E strain of RPr, which keeps a residual virulence and is used as a live TF vaccine.

Thus, according to the findings of our laboratory which performed mass inoculations with the live E vaccine, it was revealed that, at the immunization of collectives which are serologically negative to tests with RPr antigen, there is an almost general infection with rickettsias of the E strain which is expressed in their acquisition of positive CBR and HAR. At the same time, the so-called delayed reactions to vaccination, manifested by fever of some duration, headache, general malaise, and in some cases also by the presence of roseolar eruptions which appear after an incubation of 9-10 to 14-18 days and are the clinical form of vaccinal TF are observed in approximately 10%-11% of the subjects who were seronegative before inoculations. Thus, with a general infectibility of the collective, vaccinal TF "ailments" are seen only in a limited contingent of the people. It can be hardly doubted that in the given case the vaccination reveals people who are the most predisposed to the ailment of TF, and are the most resistant to it (Figure 23).

Авторы	Возраст обследованных (в годах)	31-40		41-50	
		число обследованных	% положительных по РСК	число обследованных	% положительных по РСК
Л. Н. Астахова, М. В. Матвеева, С. П. Сатуновская, Н. А. Немшилова		356	9,5	350	17,8
Г. Р. Газизова		207	16,0	123	25,2
В. А. Яблонская		244	25,4	222	30,2
Г. Ф. Долгов		374	16,0	152	25,0
Д. Ахмедов		215	16,0	162	23,0
Р. Г. Дюйсалиева		457	12,3	359	13,4
		527	14,4	429	14,9

TABLE 23

a...Authors; b...Age group of examinees (in years); c...number of examinees; d...% of positive cases by CBR; 1...L.N. ASTAKHOVA, M.V. MATVEEVA, S.P. SATUNOVSKAYA, N.A. NEMSHILOVA; 2...G.R. GAZIZOVA; 3...V.A. YABLONSKAYA; 4...G.F. DOLGOV; 5...D. AKHMEDOV; 6...R.G. DYUISALIEVA.

In agreement with this, there are also the results of mass sero-immunological examinations of the population with CBR using an RPr antigen at places where in the past TF outbreaks occurred, and subsequently in retrospect positive findings were displayed in percentages which clearly and very much exceed the actual TF morbidity rate of the population. This is convincingly shown in Table 23 where the results of CBR examinations are quoted for older and elderly groups of population (from 31 years up to 50 years of age) in different rayons of the USSR.

It is perfectly evident that the retrospectively established column of high percentage of positive CBR findings in older and elderly age groups, as quoted in Table 23, which varies within the range of 9.5 - 12.3 and 25 - 30.2%, can be explained only by a considerable dissemination of obliterated and subclinical forms of TF infection in the past.

As it has been already noted in the chapter on the clinical picture of TF, the question about the obliterated and subclinical forms of TF infection, associated with TF outbreaks, attracted the attention of the research workers already long ago, and it was answered in a favorable sense by many competent specialists of foreign countries.

We already said that the hereto pertinent literature data found their place in a well known treatise on epidemiology written by the French epidemiologists LOPTE and DE LAVERNE (1927).

Independently from the foreign authors, our domestic specialists also arrived at similar conclusion about the presence of obliterated and subclinical forms (asymptomatic forms) of TF on the basis of clinical, epidemiological and some laboratory examinations in the 30-ies. A detailed summary of these is found in the monograph of V.A. BARYKIN which was published in 1932. In this monograph, the hereto pertinent researches of the collaborators of V.A. BARYKIN, S.M. MINERVIN, A.A. ZAKHAROV, A.I. KOMPANEETS and others, are quoted in detail, as well as a number of collaborators of the Moscow Mechnikov Institute, including F.G. BERNHOF, V.H. KUTEISHCHIKOV, E.M. DOOSER and others.

The foreign and domestic authors, who in the 30-ies had only very relative methods at their disposal for detecting TF infection (WEIL-FELIX reaction, inoculation and guinea pigs under their temperature control), could not exhaustively answer the question about the obliterated and subclinical forms of TF infection, although essentially they were correct in their conclusions. In the groups of the already earlier quoted contemporary authors who confirmed the existence of obliterated or subclinical forms of TF infection which could be accurately diagnosed by serological methods, we should mention again COMBIESCU, ZARNEA, and collaborators, in Romania (1957 - 1958); FOX, MONTOYA and collaborators in North and Central America (1959); MEGOY in England (1958); ABDUL-MESSIGI in Egypt (1960); K.N. TOKAREVICH and his collaborators as well as P.F. ZDRODOVSKII and his collaborators in the USSR, and others.

In addition to the above outlined general prerequisites which result from the analysis of immunological and clinical reactions to the introduction of live vaccine and from the analysis of the retrospective detection of serologically positive contingents at mass examination of the population, data which with full probability support the existence of the obliterated and subclinical forms of TF infection, we have at our disposal a number of direct observations on the detection of these forms at the foci of TF in presence of a carrier.

In this respect, extremely demonstrative are the numerous, very accurate observations of Romanian authors, headed by the now deceased TF specialist, Prof. COMBIESCU. Let us give some examples of the subclinical forms of infection at TF foci which he and his collaborators detected.

1. In a 16-year old patient, the diagnosis of TF with delay was established by CBR in a titre of 1:512. Seven persons were in contact with the patient; they belonged to the age groups of 14-18 to 36-48 years. At their examination, positive CBR was found in 6 persons in titres of 1:128, 1:64, 1:28, 1:32, 1:256 and 1:16. After isolation of the patient, no sickness cases were observed in spite of the presence of diagnostic titres in the contacts.

2. In the second case, a clinically manifested and a subclinical TF were detected at repeated examinations. In the patient the CBR was positive at the following times: 25 June...1:1024; 22 July...1:1024; 30 July...1:1024, and 4 October...1:128. In the contact, a person who did not get sick, the results of progressive examinations were the following: 20 August...1:512; 29 July...1:1024; 4 October...1:100, and in April of the next year 1:128.

3. Focus of KUCHA-VODA. During January-February the local outbreak of TF resulted in 25 sickness cases; half of the patients were school children. At the beginning of March, CBR serological examinations were made in 168 persons who were in contact with the patients, including 33 school children in the age groups 7-14 years, and 135 persons outside the school in the age group of 15 years or more.

As a result, out of 33 school children in 14 a positive CBR was found, including 3 who had it in titres 1:128 and 1:256. Among the remaining 135 persons, positive CBR was found in 35, including 4 with titres 1:128, 1:256, 1:2048. The exposed sero-positive subjects remained healthy.

4. According to summary data, at 46 epidemic foci 2108 serum tests were examined in people who were in contact with TF patients, and clinically were not sick. In 947 cases, i.e., in 44%, the CBR was found positive, including 9% in children up to 6 years of age, and up to 53% in adults in the age groups of 51-60 years. Here, in 26 cases, the CBR was found in titres

of 1:128 (14 persons), 1:256 (6 persons), 1:512 (3 persons, 1:1024 (1 person) and 1:2048 (2 persons).

Similar findings were also described by domestic investigators.

Thus, according to the data of G.I. GRENAUS and co-workers (1960) at a focus of TF in patient P, with marked clinical TF, at the serological examinations on 30 January, a positive CBR was detected in a titre of 1:1280, and a HAR in a titre of 1:4000, and in a healthy contact of P, on 6 February the CBR gave a titre of 1:40, and the HAR a titre of 1:4000.

At another focus, in patient K, with clinical TF, on 13 January with a negative CBR simultaneously a very strongly positive HAR was detected in a titre of 1:64,000, giving later the corresponding titres of 1:640 and 1:128,000. The contact of this patient, called B., carried a febrile ailment without calling for medical aid, with a CBR titre of 1:160 and 1:8000. In contact B, there was evidently a mild form of TF infection.

Similar observations were published by M.A. KULEMINA, and K.P. KUZNETSOVA (1960) who, at the examination of 945 healthy subjects from foci and from areas where TF existed, found a positive CBR in 180 cases, including 17 persons, with titres from 1:320 to 1:1280 (13 patients) up to 1:256 - 1:2048 (in 4 patients).

In the quoted observations of the Romanian and domestic authors only cases with marked serological reactions are quoted with definitely diagnostic titres. Thus, in the serologically detected, infected subjects there was undoubtedly a very light, obliterated or subclinical form of TF infection.

All these data-----past and present-----do not justify the ideas of some domestic authors who according to a formed tradition continue to deny the existence of obliterated and subclinical forms of TF infection (L.V. GROMASHEVSKII and collaborators, 1947, 1961).

For the history and foundation of the idea on the absence of obliterated and asymptomatic forms in TF among the leading groups of Soviet epidemiologists the following should be mentioned. In his Manual on Special Epidemiology (1947), L.V. GROMASHEVSKII, stated: "In diseases which have a wide polymorphism among their mildest running ("obliterate") forms, it can be expected to find such conditions when any sickness reaction to the infection is lacking on the part of the organism. Such a condition is diagnosed as relatively healthy (infection carrying). And, on the contrary, in infections which always run in a clinically manifested form, and do not make an "obliterated course", the possibility of a healthy infection-carriage is excluded". (p. 124). Furthermore, starting out from their own observations according to which the length of the fever period in TF is never less than 9 days (minimum time, which only 2.1% of the sickness cases have), L.V. GROMASHEVSKII concludes: "This fact is important to a high degree for the clinical picture (diagnosis) and epidemiology, unquestionably repudiate the possibility of the existence of healthy infection-carriage in TF; because a disease whose easiest form which is encountered runs in the shape of a 9-day fever cannot run in the form of an asymptomatic healthy carriage" (p. 421). The indicated thesis of L.V. GROMASHEVSKII entered entirely into the official codex of TF epidemiology which in 1939 was confirmed by the All-Union Conference of Microbiologists, Epidemiologists and Infectious Disease Specialists. This Conference, according to the report of L.V. GROMASHEVSKII, considered it unfounded "...what was predominant lately in the domestic literature on the virus carriage of TF". From this time on, the question on obliterated forms and on virus carriage in TF was referred to the category of harmful epidemiological errors which should deserve the most severe criticism,



which, under the egis of L.V. GROMASHEVSKII, inspite of the accumulated contemporary data, until our time is supported by a group of epidemiologists who share his opinion (M.N. SOLOV'EV, I.I. ELKIN, I.I. SHATROV and others).

How the absence of obliterated and subclinical forms in TF was established, can be clearly seen from the below quoted work of N.I. MOROZKIN (1940), the collaborator of L.V. GROMASHEVSKII. Studying the TF outbreaks in two villages G. and Gl. with systematic thermometric observations of their inhabitants, both in the families where TF occurred and outside the families, the author found numerous cases of subfebrile conditions, including long-lasting conditions. Thus, in village Gl, 76 cases of subfebrile conditions were found lasting 5 days or more, and in village of G. there were 31 cases lasting from 4 to 8 days, including 28 cases among children. How the observed subfebrile conditions were classified clinically in the TF epidemic, is illustrated by the example of village Gl. where the following was diagnosed:--- in five persons...grippe; in 19 persons...catarrhal pneumonia; in 7 persons... diseases of the nasopharynx; in 11 patients...tuberculosis; in 12 patients... malaria; in 3 patients...otitis; in 2 patients...colitis; in 6 patients... gynecological diseases; in 5 patients...furunculosis; in 3 patients...exophthalmic goiter; and in 3 patients...lymphadenitis. Having noted that the Weil-Felix reaction did not give any conclusive results, in the subfebrile subjects and a transition of the subfebrile states into clinically manifested TF was not observed, the author came to the conclusion in summary:---"The subfebrile states never are the manifestation of atypically running TF infection, whose lightest forms are sufficiently manifested for the diagnosis of the clinical picture".

Since the quoted work refers to 1940, the author can be reproached only for categorizing his conclusions, but from the view of point of the modern methods of detecting a TF infection this work is not reliable. By all means, its author would be condemned even by the official instructions which require the obligatory use of specific sero-diagnosis in such clinical forms as grippe, catarrh of the upper respiratory pathways, pneumonia, and others---clinical diagnoses under which, as we know at present, TF infection is not unfrequently hiding.

On such and similar grounds, a tradition was created which excludes the fact presently widely accepted by specialists of various countries in regard to the existence of obliterated and subclinical forms of TF infection on the nature of which at its July 1963 session, the Committee of Experts of WHO brought the following conclusion on the ground of the presented materials:---"Examinations of the population in districts endemic in regard to RPr show that inapparent (obliterated) forms of infection are produced by these agents rather more frequently than it is thought. Further information is desirable on the frequency of these forms of infection and their possible role in the maintenance of the infective cycle".

Even though at present the researchers who are not burdened by tradition do not doubt the existence of obliterated and subclinical forms of TF infection, the question about the epidemiological significance of these forms still remains unclear. As we know, in all light forms of TF, the infectiousness of lice for the patients is insignificant. Evidently, in case of the obliterated forms of TF infection, these relations can be also similar. It is obvious that the epidemiological importance of the obliterated and subclinical forms in TF should not be overestimated, but at the same time it should not be underestimated either. Further observations are needed on this problem.

TF epidemics arise only in the presence of pediculosis of the population, and the territorial spread of it is proportionate to the movement of

lously and infected subjects or groups of the population. Hence, it is generally known what the relationship is between epidemic TF and the low sanitary living conditions of the population, and the special predisposition of the population to mass TF sickness in time of social disasters and catastrophes (wars, famines, and so on). The timing of TF outbreaks at the cold winter-spring (January-May) in its turn finds its explanation in the characteristic density of population in this season, and the temperature optimum (about 30°) for lice under the warm clothing which is worn in the cold weather (G.S. MOSING) and the deterioration of the nutrition of the population (lack of vitamins and so on).

In local outbreaks (e.g., family outbreaks), when conditions are particularly favorable for the dissemination of infection (pediculosis, density, lack of hospitalization of patients), the TF morbidity rate can be very general. But even with long lasting epidemics, in case of extraordinary wide dissemination of TF infection, it never afflicts all the susceptible population (L.V. GROMASHEVSKII). Thus, in the epidemic of 1919 - 1923 in the USSR, according to the official data, up to 10% of the population suffered from TF, with a 25% affliction admitted by some authors (L.A. TARASEVICH).

Depending upon general conditions, the duration of a TF epidemic can vary in a very wide range---from some weeks to several years.

On the question of maintenance of the TF virus in the interepidemic period, different hypotheses were announced:

1) The hypothesis of MOOSER on the rat production of TF outbreaks. According to this hypothesis, in the presence of pediculosis of the population, it is assumed that the R. Mooseri can change, i.e., the agent of rat rickettsiasis can change to R.Pr as a result of subsequent passage of the R. mooseri through lice which are infected on patients with rat TF. This hypothesis, actually supported by several Japanese authors (KODAMA, OKAMOTO, et al.,) had however no experimental (E.F. EPSTEIN, 1958) or epidemiological confirmation, and it was abandoned by its authors themselves.

2) The hypothesis of survival of the TF virus in places of its epidemics in fleas which are infected on TF patients (F. BLANC, and M. BALTAZARD, 1941). Although in the experiments fleas are relatively easily inoculated with RPr, this hypothesis contradicts direct epidemiological observations (CH. NICOLLE, and collaborators) in absence of findings of the infectiousness of fleas with TF rickettsiae under natural conditions.

3) The hypothesis of Polish authors on the possibility of long preservation of RPr in dead lice and in their fecalia in the outside environment (STAZHIK, 1936, 1948). Based upon experiments which were confirmed by a number of authors (see the proper chapter) this hypothesis can explain at full satisfaction individual episodes of TF and their outbreaks (infection through the clothes of TF patients which are contaminated with the fecalia of lice), but it could not explain the problem of preservation of the TF virus in the extraepidemic period.

4) The hypothesis of REISS-GUTFREUND (1955-1956) with the findings of rickettsiae in Abyssinia in the interepidemic period in domestic animals and in several other types of ixodide ticks. These data were however not corroborated by anybody, and it is difficult to evaluate them.

5) The hypothesis of recurrent production of repeated TF sicknesses which in presence of pediculosis could be the source for the inoculation of lice and dissemination after their infection. This hypothesis is based upon the assumption of ZINSSER (1936) that latent forms of TF infection exist in the

previously infected and sick persons, and their clinically manifested aggravation (exacerbation) is possible in the form of a "second" TF (Recurrent TF, or Brill's disease). This hypothesis is corroborated by PRICE (1955) with the findings of RPr in the lymph nodes in two subjects who in the remote past, passed through TF. At the present time the hypothesis attracts greater attention and it is accepted by many specialists. To this hypothesis we will return somewhat later.

6) The hypothesis of uninterrupted character of infections according to which in the interepidemic period the TF infection is supported by chains of individual disease cases which are spread by lice, but not infrequently they escape from the epidemiological record. Here it is considered that in the preservation of the TF infection children may play not a small part in whom, as it is well known, TF runs in a mild form, and therefore, it is overlooked. The latter statement is however not corroborated, since in case of sporadic ailments of TF the serological indices of past infections are characteristically absent in children in the interepidemic period. As in the previous case also (the recurrent hypothesis), to this hypothesis we shall also return at the discussion of the epidemiology of sporadic TF.

These are the more important data for the characterization of the epidemiology of epidemic, louse-borne or classical typhus fever.

Let us go over now to the epidemiology of the sporadic forms of TF.

#### SPORADIC TYPHUS FEVER OR BRILL'S DISEASE.

As it was shown in the section on the clinic of TF, under sporadic TF a clinical epidemiological variety is distinguished which is etiologically identical with epidemic TF, but differs from it by a number of special features which include: ---the episodic nature of singular sickness cases, as a rule in absence of pediculosis and with impossibility to recognize the source of infection; a mild course of the disease in presence of symptoms which are attenuated, though characteristic for TF, including an accelerated course of the disease; relative difficulty to isolate RPr from the blood of patients; presence of well-manifested serological reactions in the patients when RPr antigen is used, but with frequent failure of the Weil-Felix reaction which is characteristic for epidemic typhus fever; occurrence of sickness cases in elderly and older age groups; lack of any marked seasonal character; finally, sporadic TF is found among people who in the past experienced outbreaks of epidemic TF, and among people who emigrated from places which were afflicted with epidemic TF, into districts which are free of this infection.

In the described characteristic form, sporadic TF is equivalent to second (recurrent) TF which West-European and American investigators identified with the disease of BRILL-ZINSSER.

With the analogous clinical epidemiological characteristics, sporadic TF can be also recorded in people who deny TF sickness in the past, which does not exclude, however, that they actually have a second (recurrent) TF sickness, since in these people TF could have taken an atypical course which remained undiagnosed (see above). In every case such a stipulation, which widens the category of persons sick with sporadic TF as a recurrent case of sickness, is completely probable, and not without reason some authors (K.N. TOKAREVICH, 1958) emphasize the complete absence of modern data on the spread of atypical and obliterated forms of TF. Hence, in our opinion, the term "sporadic TF" is preferable to the term "recurrent TF", since the latter denomination is determined entirely by anamnestic data, i.e., by a criterion of relative reliability. However, it must be taken into consideration that a "sporadic"

case can be also an imported TF sickness case of epidemic origin. But evidently, even in this case a thorough clinical epidemiological analysis can differentiate in nature of "sporadicity" of a sickness case, with the consideration of the whole complex of the above mentioned symptoms.

In their most essential features, the indicated general characteristics of sporadic TF are specified in the following manner.

As already indicated above, as to its etiology sporadic TF is identical with epidemic TF, i.e., it is caused by RPr, which was established by previous (ZINSSER, CASTANEDA, 1933) and modern observations (MURRAY, SNYDER, KOSTSHEV-SKII, G. S. MOSING, F.I. KRASNIK, 1950 - 1958). At the same time, for sporadic TF characteristic is a relatively small concentration of the pathogenic agent in the blood of patients. Hence, it can be isolated considerably less often and with more difficulty than in epidemic TF. Thus, according to the data of G.S. MOSING (1952), at repeated blood sucking the infectiveness of lice was only 1%-5% in sporadic TF, whereas in epidemic TF already after a single half-hour feeding of the lice 10%-40% became infected. In similar observations of F.I. KRASNIK (1958), when feeding lice on the 5th-8th day of sickness, their infectiousness was established in 14%-44%. Here, in all 14 cases of positive findings, the RPr were detected only in subpassages in the inoculated lice in sporadic TF.

According to verbal information received from Romanian authors (ZARNEA, BUZDUGAN, 1963) and according to Yugoslav observer (J. GAON, 1963), in recent years they were generally unable to isolate RPr from patients with sporadic TF. Observations in our laboratory are in full agreement with the quoted data. Thus, in 132 experiments of inoculating lice according to different methods (inoculation with blood through a microenema according to WEIGL, feeding blood through chick membrane, the blood sucking on the skin of sporadic TF patients), only a single case, i.e., less than in 1%, was it possible to isolate RPr by culture (G.M. DUTOVA, V.F. IGNATOVICH).

In view of the unquestionable identity of the etiology of epidemic and sporadic TF, the problem about the level of virulence of RPr strains isolated from sporadic TF patients is evidently still not clarified, since the pertinent investigations were limited to the detection of the typical nature of the isolated cultures of rickettsiae. In our opinion, however, the formulation of the question about the virulence of RPr in sporadic TF which runs in mild forms of sickness, is completely in order, and justified also by the data accumulated on the phenomena of variability in the group of rickettsiae in general, and of Rickettsiae prowazeki in particular. Suffice it to point to the well known fact of spontaneous split off of the vaccine Strain E of RPr from the virulent TF culture "Madrid". According to the data of our laboratory, when we studied the laboratory collection of different RPr strains by a special methodology, a marked diversity of their virulent properties was distinctly revealed (V.F. IGNATOVICH), not to mention that strains of unusually common characteristic are isolated from TF patients which in our laboratory were labelled as variants, intermediate forms between RPr and R. mooseri (R. prowazeki var. intermedia, E.M. GOLINEVICH, 1953).

However, regardless of this, the available observations clearly established that in presence of pediculosis, cases of sporadic TF can be the source of epidemic outbreaks of TF (K.N. TOKAREVICH, and F.N. KRASNIK, 1958; M. STOLZOVA-SUTIROSOVA et al., 1959; and so on). Therefore, no liberal attitude is permissible in the control measures in regard to sporadic TF, especially in its mildest forms.

As it was already repeatedly mentioned, for sporadic TF characteristic is a mild course, with a shortened febrile period in the average limits of

9-11 days, with possible shortening to 4-6 days, with a generally favorable course of the disease. In epidemic respect, it is very important to emphasize here that with its frequency of atypical course, sporadic TF is often recorded under mistaken diagnosis (grippe and grippe-like conditions, catarrh of the upper respiratory pathways, pneumonia, paratyphoid, abdominal typhoid, and so on), and in such cases it can be diagnosed only by serological examinations. Corresponding illustrative examples were already given in the preceding sections of this book. Returning to this question, we remark only that, according to the findings of Romanian authors, under the above indicated labels erroneous diagnosis of sporadic TF was noted in 60% of the cases, while in recent years, as we have already seen, about half of the sporadic forms of TF generally were diagnosed only serologically (COMBIESCU et al., 1959; ZARNEA, BUZDIGAN, 1963). Let us also remark that, according to the findings of K.N. TOKAREVICH and F.I. KRASNIK, precisely from such cases of undiagnosed TF, in the presence of pediculosis, group ailments arose which they described.

As we have seen already, for sporadic TF characteristic is a serological diagnosis of the ailment only by reactions which use an antigen made of RPr, while the WEIL-FELIX reaction is not efficient, though it is well marked in epidemic TF. Remembering this in the epidemiological survey, let us give a very characteristic illustration of the mentioned fact from the published findings of Romanian authors (COMBIESCU, ZARNEA et al, 1959) which are shown in Figure 28.

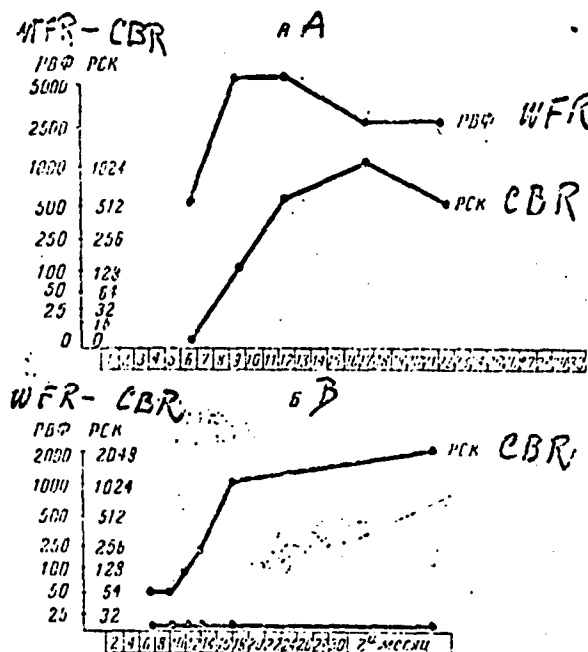


FIGURE 28. - Complement binding reaction (CBR) with a RPr antigen, and WEIL-FELIX reaction (WFR) with Proteus OX<sub>19</sub> in epidemic (A) and sporadic (recurrent) (B) TF (according to COMBIESCU and collaborators, 1959).

For sporadic TF, it is characteristic that it appears after a TF epidemic, being a sort of consequence of the latter. Moreover, in presence of a practical elimination of outbreaks, sporadic sickness cases set in with

marked constancy at a certain level, with slight annual variations, if the control of pediculosis is systematically conducted. An illustration of this is found in the statistic of TF in Poland during 1945-1958, in presence of a postwar epidemic in 1945-1946. The pertinent data are given in Table 24, with calculation of the TF sickness cases per 100,000 inhabitants (KOSTSHEVSKII and VOITSEKHOVSKII, 1959).

For the epidemiology of sporadic TF, together with the individual nature of sickness cases, admittedly specially characteristic is the impossibility to determine the sources of infection in absence of pediculosis. Let us give a suitable illustration from foreign sources.

Таблица 24

а Год	б Число больных сыпным тифом	в Заболелость на 100 000 населения	д Примечание
1945	15808	66,1	1945—1946 гг. — эпидемический период с преобладанием эпидемических форм заболевания до 1949 г. На протяжении 1950—1953 гг. — преобладание спорадических форм. Начиная с 1954 г. — спорадический сыпной тиф с незначительным процентом групповых заболеваний. В 1953 г. в Польше повсеместно введена серодиагностика сыпного тифа по РСК, с чем связано «повышение» заболеваемости (улучшение диагностики).
1946	3118	14,7	
1947	535	2,2	
1948	391	1,6	
1949	389	1,6	
1950	214	0,9	
1951	211	0,8	
1952	219	0,9	
1953	350	1,3	
1954	369	1,4	
1955	392	1,4	
1956	433	1,6	
1957	369	1,3	
1958	287	1,0	

TABLE 24. - a...Year; b...number of TF patients; c..Morbidity rate over 100,000 of population; d...Remark; e...1945-1946---epidemic period with predominance of epidemic forms of the disease until 1949. During 1950-1953, predominance of sporadic forms. Beginning from 1954,--sporadic TF with a small percentage of grouped cases. In 1953, in Poland everywhere the CBR serodiagnosis of TF was introduced, and this explains the "increase" in the morbidity rate (improved diagnosis).

Thus, Polish investigators, being anxious to keep the TF sickness cases at a certain level, inspite of the introduction of broad control measures throughout a number of years, in 1955 conducted a detailed analysis of 853 cases of TF recorded during three years (1952-1954). Here it was revealed that in 1952 the epidemic form of sickness cases amounted to 16% of the total number of recorded cases, and in 1954 they amounted only to 5.9%. Thus, in 1954, 94.1% of the sickness cases were referred to sporadic TF; moreover, in 70.8% of all cases in this year the possibility of an exogenous contact was completely excluded, while in a group of sporadic TF, the sources of infection were excluded in 95.1%, with complete absence of the louse factor (KOSTSHEVSKII and VOITSEKHOVSKII, 1959).

Similar was the evolution of TF also in Romania where there was a severe epidemic during 1944-1948, on account of which, under the influence of the measures taken, epidemic forms gradually were replaced by sporadic forms which, at the special investigations during 1953-1956, showed the following relations:---in 1953, primary TF was recorded in 80% of the cases, and sporadic TF in 20%, while in 1954 these ratios were characterized by the respective figures 53.6 and 46.4% (COMBIESCU et al, 1959). Further on, this change of forms continued, and during the subsequent two years (1961-1962), in the Suchava

Oblast which was most afflicted in the past, only sporadic (recurrent) forms of sickness cases remained, without any definite sources of infection and without a louse factor, but at the same time the level of sickness cases was maintained without noticeable changes (from the account of BUZDUGAN in Suchava, January 1963).

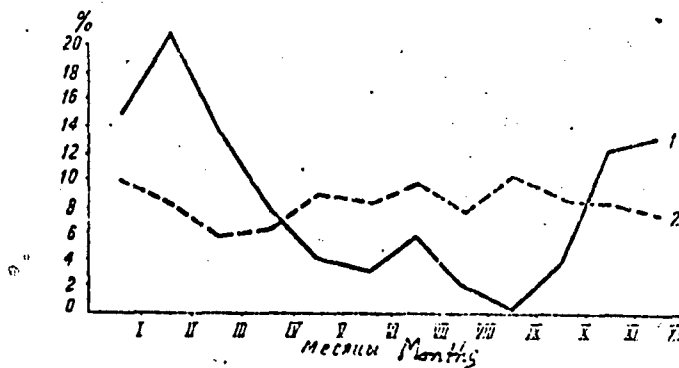


FIGURE 29. - Graphs of the spread of primary and second (recurrent) typhus fever cases by seasons in Poland (after KOSTSHEVSKII and VOITSEKHOVSKII, 1959). 1...epidemic typhus fever in 1953-1957; 2...recurrent (second) TF in 1953-1957.

In difference from epidemic TF, for sporadic TF characteristic is the absence of a seasonal character. This thesis is clearly illustrated by the below given graphs of epidemic and sporadic (recurrent) TF, which were borrowed from the already repeatedly quoted survey of KOSTSHEVSKII and VOITSEKHOVSKII for Poland (Figure 29).

Parallel with the absence of a winter-spring seasonality which is typical for epidemic TF, for sporadic TF particularly characteristic are the shifts of morbidity rate to the older and elderly age groups, with a practical absence of morbidity among children of the preschool and school age, and with a relative rarity of sickness cases among young contingents in general, i.e., in those age groups which are afflicted by epidemic TF. These relations of the morbidity rate of the population suffering from sporadic and epidemic TF in an age-wise cross section are especially clearly demonstrated in the below given graphs of domestic (Figure 30), Polish (Figure 31) and Romanian (Figure 32) authors.

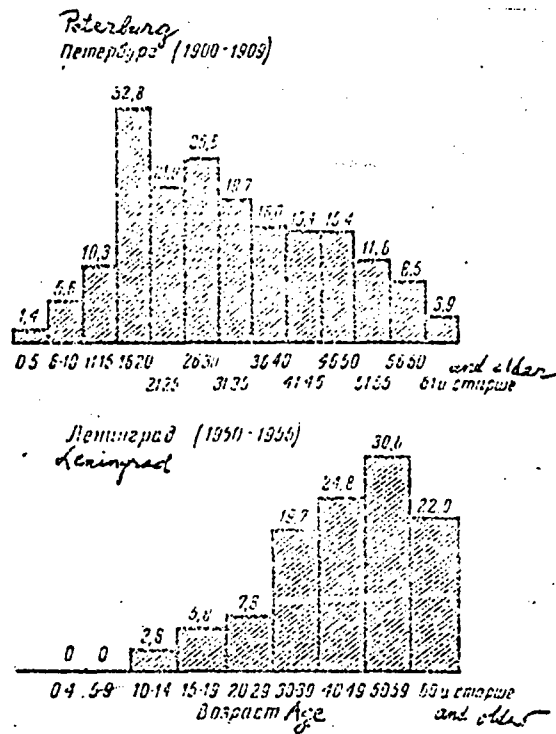


Figure 30. - Diagram of age distribution of cases of epidemic (Peterburg) and second (recurrent) (Leningrad) typhus fever (average morbidity rate per 100,000 population (after F.E. KRASNIK).

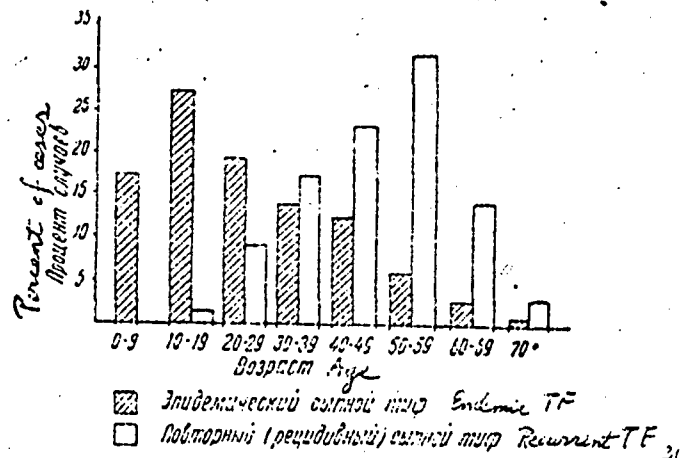


Figure 31. - Diagram of age distribution of sickness cases of epidemic and second (recurrent) TF in Poland (after KOSTSHEVSKII and VOITSEKHOVSKII, 1959).



The quoted data are in full agreement also with domestic data. Thus, according to the statistics given by K.N. TOKAREVICH (1958), in the 50-50 year age group the index of morbidity rate due to sporadic TF per 100,000 population is lately 10 times higher than the same index for the 10-14 year age group. In agreement with this, according the already earlier quoted long-range material of M.M. FIGURINA and A.N. SEMENOVA, among the hospitalized patients who had sporadic TF, almost 60% belong to the age group older than 45 years; moreover 14% is in the 60-78 year group.

Specially interesting is the comparison of age-wise morbidity rate due to sporadic TF with the picture of the immunological structure of the population in age-wise cross section, as this is shown at present at the mass serological examinations of the population with the CBR test.

Our laboratories organized mass examinations with CBR for the purpose of a retrospective detection of the immunological structure of the urban and partly of the rural population in different rayons and republics of the USSR. All these observations, made on many thousands of people, who in the past were subjected to sickness of TF of some strength, with the presence of only sporadic cases at the time of examination, gave a stereotype picture form of the age-wise distribution of immune layers, as this is shown on the attached Figures 33 - 35. With variations of the average percentage of immune layer

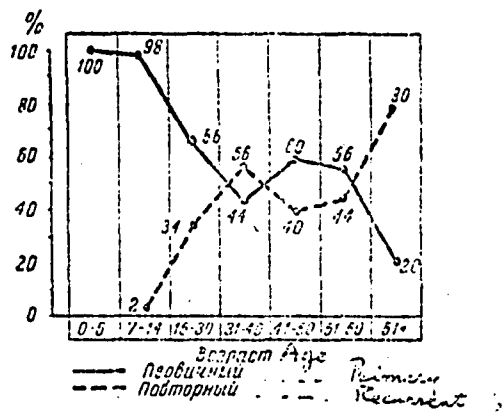


Figure 32. - Graphs of age-wise distribution of cases of epidemic and second (recurrent) TF in Romania (after COMBIESCU et al., 1959).

for all ages in the ranges from 5 to 13, all pertinent observations in accord showed an immune layer of peak strength in the elderly group (from 41 years on to 60 years and older), with a minimum strength of immunity in youth and especially in the childhood age groups. In other words, as shown by the indicated data of immunological examinations, at present the maximum susceptibility to TF infection exists in children and in the young age groups which are the least afflicted with sporadic TF, while the smallest susceptibility is in the elderly age groups of the population which are the most afflicted with sporadic TF. As this can be seen from Figure 30 and 32, the reverse relations exists for epidemic TF.

Таблица 25

1. Возраст привитых (в годах)	16-18	19-22	23-25	26-30	31-40	41-50	51-60
2. Процент положительных поздних реакций на прививку	12,2	5,4	5,5	7	4,6	3	1,6

TABLE 25 - 1...Age of inoculees (in years); 2...Percent of favorable late reactions to inoculation.

The immune layer, determined by CBR, in agreement with its level in the different age groups, actually reflects their different susceptibility to TF infection at the time of examination. This is very clearly corroborated by observations of our laboratory on the comparative age-wise distribution of delayed specific reactions to inoculation with live TF vaccine made from the E strain of RPr. The pertinent observations of V.A. YABLONSKAYA are summarized in Table 25 on the basis of the age-wise distribution of delayed reactions among 2558 inoculated persons.

From Table 25 it can be clearly seen that the age groups (41-60 years) which are the most afflicted with sporadic TF are the least susceptible to TF infection, which is shown in their reaction to inoculation with an attenuated TF virus. And, on the contrary, the young groups (16-18 years), which are rarely or not at all afflicted with sporadic TF, show the maximum sensitiveness to TF infection according to the same symptom.

The immunological structure of the population which we obtained in their age-wise cross section, related to the places where sporadic TF cases exist, in an epidemiological respect also give a very valuable index:--- They objectively attest that at the places of examination active sources of TF infection and factors of its spread are absent,---otherwise, the reason for the lack of morbidity in the young age groups which are the most sensitive to the infection cannot be understood. And that these younger age groups do not get sick and do not become infected, is objectively shown by their negative serological examinations which for them prove the absence of any contact with TF virus (Figures 33, 34 and 35).

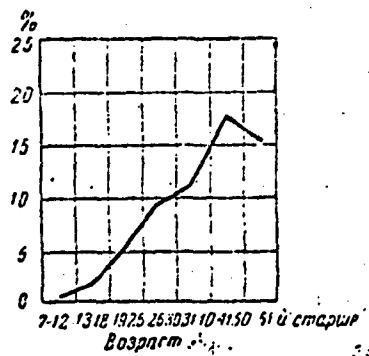


FIGURE 33. - Summarized graph of age distribution of retrospective positive CBR with a RPr antigen, obtained at group examination of the healthy population (5124 persons) of five towns (after P.F.ZDRODOVSKII).

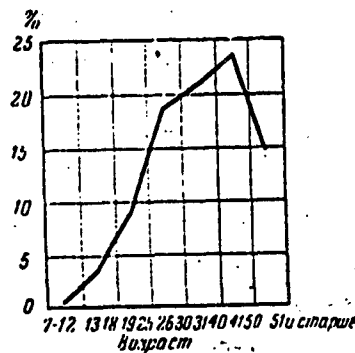


FIGURE 34. - Graph of the age distribution of retrospective positive CBR with a RPr antigen, obtained in group examination of healthy population (1182 persons) in one of the towns (After V.A. YABLONSKAYA)

These are the more important epidemiological features of sporadic (second) TF.

On the nature and the origin of the described forms of sporadic TF, two hypotheses are in existence:---the hypothesis of its recurrent or endogenous origin, and the hypothesis of its louse-borne or exogenous origin.

First of all let us remark that both hypotheses unanimously recognize the fact of a reduction or loss of immunity after a previous TF, to which its recurrence is also related.

The very fact of a second, and in some cases of a third TF infection proves convincingly that immunity acquired by a TF infection can weaken or it can disappear.

Lately, we had seen a sufficient number of cases of a sort of experimental detection of the loss of immunity after TF. Thus, in making mass inoculations with live TF vaccine, after a 16-18 day incubation a marked delayed reaction was seen in 3 persons who had had TF infection 14, 40 and 42 years before (V.A. YABLONSKAYA, 1962). In people who are immune to TF with a positive CBR, this kind of delayed reaction was not met with.

Let us turn now to the analysis and evaluation of the above mentioned hypotheses.

The hypothesis of the recurrent origin of a second (sporadic) TF was first formulated in 1934 by ZINSSER in view of sporadic cases of TF observed in the USA among emigrants from TF endemic places of Eastern Europe, and described by BRILL in 1916. After confirming with CASTANEDA (1933) the TF etiology of Brill's disease, and having studied the epidemiological findings, ZINSSER came to the conclusion that it was a clinically manifested exacerbation (recurrence) of a latent infection after a previously suffered TF. Further on, chiefly on the basis of analogy with some rickettsias in animals, ZINSSER's hypothesis as to the second TF found confirmation by French investigators in Algier (L. & G. PARROT, 1939).

In 1955, the recurrency hypothesis of the origin of second TF had its corroboration in the already earlier quoted work of PRICE who in two subjects of positive CBR, who suffered earlier from TF and were not in contact with TF

patients for a period of 6 years, isolated RPr from their inguinal lymphnodes, having used for this purpose a special method for the preliminary enrichment of the lymphoid tissue with rickettsias. It should be added here that with similar isolations of rickettsiae the presence of chronic latent infections was shown in case of Rocky Mountain spotted fever (PARKER, et al., 1954), and in tsutsugamushi fever (J. SMADEL et al). Latent forms of infection are met with also in Q-fever, and they were known long ago for Wolhynian fever (G.S. MOSING et al), In one word, the possibility of latent infections was shown practically in all more important human rickettsias.

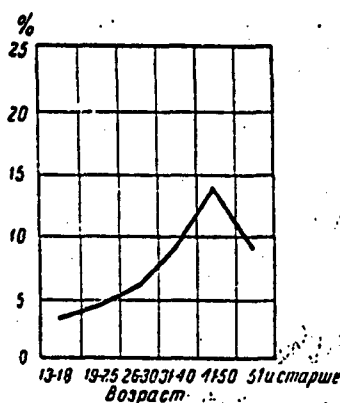


FIGURE 35. - Graph of age distribution of retrospective positive CBR with antigen made from RPr, obtained in group examination of healthy people (1557 persons) in one of the towns (after L.N. ASTAKHOVA and others).

In addition to this, it should be also added that, as this was already explained in the chapter on the serology and serodiagnostics of rickettsias, in the USA in emigrants with an anamnesis of TF in the past, among whom sporadic TF cases were observed, in a considerably large percentage (up to 30%) of the cases, positive serological tests were found with an antigen made from RPr (MURRAY, SNYDER, PRICE and others).

There is no direct experimental foundation of the hypothesis of recurrences in TF, since in difference from rickettsias with a natural focality, latent types of TF infection cannot be regularly reproduced in animals, being limited only to episodic observations of lingering forms of this particular rickettsiasis in cotton rats. At the same time this type of latent infection, with an indefinitely long maintenance in the brain, can be regularly observed in white mice which are afflicted with *R. mooseri*, closely related to RPr and very probably being the predecessor of the latter in the past.

According to observations in our laboratory, in rats infected with *R. mooseri*, one year after the infection when the pathogenic agent is found only in the brain, under the effect of cortisone and in conditions of stress (cooling), a generalized infection is produced in a certain percentage of cases with the appearance of the pathogenic agent in the blood, which to a certain extent is a model for recurrent latent rickettsial infection (V.A. YABLONSKAYA, 1963).

Applied to TF, the quoted experiments have however the value of only a more or less reliable analogy.

By evaluating the recurrency hypothesis in comparison with the above exposed epidemiological peculiarities of sporadic (second) TF, its validity should be objectively recognized, since from the viewpoint of the mentioned recurrency hypothesis, all peculiarities which are singular to TF can be explained without difficulty and with full reliability.

In this regard, particularly convincing are the data on the preferential concentration of second TF particularly in the older and elderly age groups where, as we have seen, the maximum immune layer is concentrated--- in view of the reverse conditions which are shown by epidemic TF afflicting the most sensitive younger and young age-group contingents (See Figures 30 - 32). The conclusion is therefore completely logically founded that the paradoxical concentration of sporadic TF in the older and elderly age groups of the maximum immune layer, reflecting their affliction with TF infection in the past, is due to the endogenic origin of sporadic (second) TF. In its turn, absence of TF in the most susceptible younger age groups indicates absence of exogenous sources and factors of TF infection or their very weak presentability, which is in agreement with the recognition of the endogenous nature of second TF, as considered by the recurrency hypothesis (Figure 36).

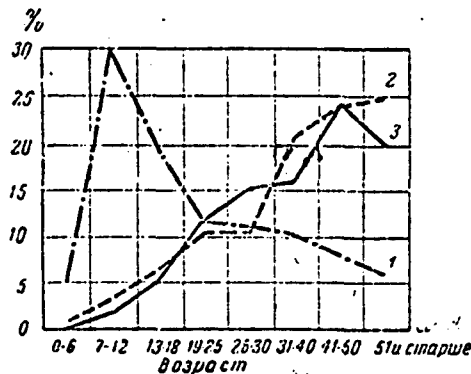


FIGURE 36. - Graphs of age-wise distribution of cases of epidemic (1) (war time), and sporadic (2) (postwar years) of TF in Byelorussia, and graph of age-wise distribution of positive CBR tests among healthy (3) persons (examination of 2770 persons) in comparison with the graph of sporadic cases.

Very demonstrative are also the undoubted hospital sickness cases of TF under conditions which completely exclude their exogenous origin. K.N. TOKAREVICH describes ten cases of such ailment, by characterizing them in the following manner.

1. All these cases arose in towns under conditions of well arranged non-infectious hospitals and clinics in the absence of epidemic TF in the given locality.
2. All patients who were considered had had TF in the past.
3. TF with well manifested serology arose in them 18-40 days after their hospital-admission, i.e., under conditions which exceed not only the average but also in many cases the maximum duration of incubation.

4. In the patients the TF infection was preceded by various stresses in the form of pneumonia, surgical interventions, mental disturbances, child delivery, and so on.

5. All sickness cases remained singular.

6. The most thorough search after the sources of infection within and without the hospital remained without results.

As an illustration we quote the case of sickness in woman citizen K., 35 years of age, who had had TF 15 years ago, and was hospitalized with right-sided pleuropneumonia, and two weeks after its end she developed an acute febrile disease with scanty roseolar exanthema. On the 12th day, CBR with a RPr antigen gave positive result in a dilution of 1:3200. A serologically established diagnosis of TF was not objected to by the specialists in infectious diseases.

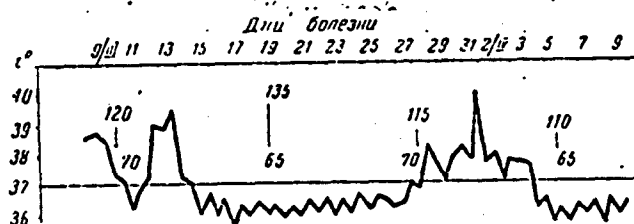


FIGURE 37. - Case of hospital recurrence of TF. Temperature graph (according to K.N. TOKAREVICH and co-workers). ---The vertical lines indicate the blood pressure.

On Figure 37, the fever graph of patient K. is presented, which showed a subsequent change of pneumonia, intermediate two-week apyrexia, and TF infection.

Finally, the likelihood of the recurrency hypothesis is well proved by the numerous cases of sporadic TF described in the literature, which were observed at places free of TF, among people, arriving from localities endemic to TF and earlier having had a TF infection.

Thus, analyzing the agewise distribution of second TF, KOSTSHEVSKII (1953), collected 954 cases of the sporadic form of typhus which were recorded in different countries free of epidemic TF. One of the pertinent and especially characteristic illustrations is a recent communication of the well-known H. DERRICK (1959) from Australia which country from 1869 on, i.e., for the period of the past 90 years, was free of typhus fever. Here, in one of the towns in 1959 a TF ailment of 10-day fever was serologically detected in an emigrant from Poland, 16 years after he had TF in the motherland.

All the above discussed, concerning the epidemiological and other characteristics of sporadic or second TF, objectively corroborates the probability of an endogenous, or recurrent origin of the sporadic forms of TF. Hence, it is natural that the recurrency hypothesis is shared by an overwhelming majority of specialists in America, Western Europe, and the democratic countries (Poland, Czechoslovakia, Romania, Yugoslavia). The recurrent nature of sporadic TF with its denomination as Brill's disease is also legalized in the nomenclature of infectious diseases, which was accepted by the World

Health Organization.<sup>1)</sup>

In the USSR, the recurrency hypothesis has been already long ago based upon extensive epidemiological and laboratory data, and it was actually corroborated by the laboratories of G.S. MOSING (L'VOV), and K.N. TOKAREVICH (Leningrad).

The recurrency or endogenous hypothesis of the origin of sporadic or second TF is opposed by the hypothesis of louse-borne or exogenous origin of the second ailments of TF. These ailments are connected with a reinfection of the people who had had TF in the past, but lost their immunity to the disease. This hypothesis found its great popularity among the group of Soviet epidemiologists (L.V. GROMASHEVSKII, M.N. SOLOV'EV, I.I. ELKIN, I.I. SHATROV and their school).

In comparison with the recurrency hypothesis, the hypothesis of louse-borne origin of the second, or sporadic TF has however little objective ground, and to a considerable degree it is maintained by earlier shaped traditions. All the above described features peculiar to the epidemiology of the second forms of TF (episodic character, lack of sources of infection and of carriers, general diversity of the age structure of sickness cases, morbidity rate of settlers who came from places where TF is endemic to places and districts which are free from TF, and so on) do not find objectively based explanations in the statement of the louse-borne hypothesis, and its defenders are in need of all sorts of assumptions.

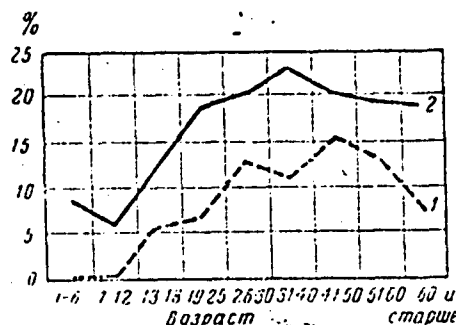


FIGURE 38. - Comparative graphs of agewise distribution of positive CBR with a RPr antigen among healthy population and on the background of sporadic (1) and epidemic (2) TF according to the data of an examination of 1837 and 2051 persons respectively (after A. TURSUNOVA).

Hence, it is natural that the recurrency hypothesis (or rather theory) acquires a greater and greater popularity, especially in the light of modern

<sup>1)</sup> FOOTNOTE: The Committee of Experts of WHO, on its July 1963 session in Geneva, having listened to the account of the author of the book on Brill's disease in the USSR, after the presented material brought the unanimous decision formulated in the following shape: "The pathogenic agent of epidemic TF can be preserved for years in recovered healthy subjects and after different intervals of asymptomatic latent infection it can cause recurrences of the disease. These recurrences can become sources of infection for lice with rickettsias. Thus, people who in the past were infected with RPr continue to remain potential sources of louse-borne TF". At the same time the Committee decided the need of further investigations to detect the causes and mechanisms of recurrence of latent TF infection.

Later on (in October 1963) the Bureau of Hygiene, Microbiology and Epidemiology Division of the USSR Academy of Medical Sciences, having heard and having discussed the same account of the book's author at an enlarged conference, accepted the well-founded possibility of the recurrent nature of the sporadic forms of TF.

sero-immunological researches which not only reliably reveal the recurrent nature of sporadic (second) sickness cases of TF, but also permit to reliably differentiate the epidemic and sporadic forms of this infection. As we have already seen, among these sero-diagnostic signs there are first of all the characteristic picture of the immunological structure of a collective, determined by CBR in the absence or negligibly small percentage of positive reactions in children not of a sick group, and with their maximum accumulation in the elderly age groups which are mainly suffering from sporadic typhus. Other ratios are observed if the immunological structure is determined on the background of current or recent ailment cases of epidemic TF, as this is illustrated in Figures 38 and 39. To the characteristic immunological structure

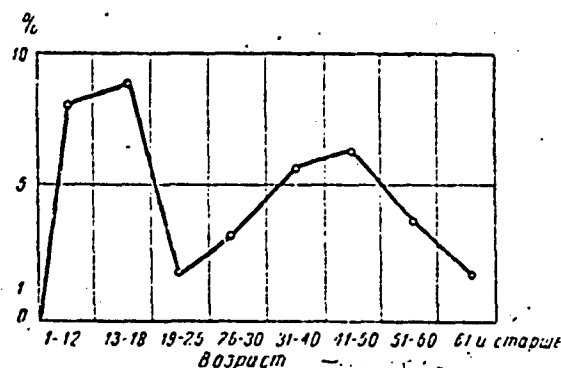


FIGURE 39. - Curve of agewise distribution of positive CBR made with and antigen from RPr, among the healthy population (744 persons examined) in one of the villages a year after an outbreak of epidemic TF.

of the population should be added the not less characteristic results of serological examinations of the environment of the patients:---in sporadic typhus even if seropositive people are found with CBR, in the environment of the patients (in absence of pediculosis), they are only with titres within the ranges of 1:10 - 1:80, i.e., titres corresponding to the retrospective detection of TF infection in the past, while in these subjects HAR is noted to be negative (in the diagnostic titre of 1:1000). Different results are obtained at the serological examination of epidemic TF foci, where, in the environment of the patients, CBR is frequently found in diagnostic titres (1:160 and higher) and HAR (in titres of 1:1000 and higher). (See Table 26).

TABLE 26

Таблица 26

	Число обследованных	% Результат по РСК, %	
		1:10-1:50	1:160-1:2500
Спорадический сыпной тиф	153	17,6	0
Эпидемический сыпной тиф	307	22,8	7,5

Примечание. Данные обследования здоровых лиц в окружении больных (по А. Турсунову).

TABLE 26 - 1...number of examinees; 2...result by CBR in %; 3...Sporadic TF; 4...Epidemic TF; 5...NOTE: Data of examination of healthy people in the environment of patients (after A. TURSUNOVA).



Finally, according to the data of MURRAY, published in October 1963 at the International Congress in Rio de Janeiro, primary TF and Brill's disease can be well differentiated by serological signs which include the following in particular:

1. Unequal stability at heating to 60° in the antibodies which produce CBR (their thermal resistance in case of Brill's disease).
2. Unequal stability to the action of mercaptoethanol in the antibodies of the agglutination reaction (resistance of the antibodies in Brill's disease).
3. Their distribution among different fractions of gamma globulins (19S in case of epidemic TF, and 7S in case of Brill's disease).

It should be however particularly emphasized that a sharp contrast of the two hypotheses is not justified by the practice at all, since, regardless of any ideas on the origin of sporadic sickness cases of TF, the measures for their control remain one and the same---absolute control of pediculosis, including pediculosis of the head (See below).

#### MEASURES OF CONTROL AND GENERAL PREVENTION.

The measures for control and general prevention of TF are carried out in correspondence with the basic thesis of modern epidemiology of this infection:---the only source of infection in TF is the sick (resp. infected) man, and the carrier of TF infection is the body louse, with the possible participation of the head lice, too, in this transmission.

In correspondence with the indicated, the entire set of measures for the control of epidemic (louse-borne) TF includes, on the one hand, neutralization of the sources of infection, which are the sick persons, and on the other hand---destruction of lice both in the primary focus of infection, and in the secondary foci, if such foci exist or are suspected.

The basic requirement of the neutralization of sources of infection means the earliest possible detection of TF patients or patients suspected for TF, with their undelayed hospitalization.

It goes without saying that the early diagnosis of TF patients is a rather important precondition for the elimination of the sources of infection connected with the patients. Unfortunately, there are no methods for an early laboratory diagnosis of TF, and the task of early detection of TF patients is solved by the competence of physicians and clinicians. This question is further complicated, since as we have already seen from the preceding material, together with the marked forms in epidemic TF, we can also find atypical ailments with mild course which are especially true for TF ailments among children. Hence, there is a general need for the hospitalization of people suspected of TF, including preventive hospitalization (or suitable isolation) of people with an indefinite fever lasting more than 5 days. Here, the volume of preventive hospitalization or isolation of patients is decided by concrete epidemiological indices (nature and size of the outbreak, dissemination of pediculosis, residential and living conditions, and so on).

In hospitalized patients, including all categories of preventive hospitalization, the diagnosis of TF is made more accurate or eliminated with modern methods of TF serodiagnosis (see the proper sections), which have a decisive importance for the correct diagnosis of TF, especially in case of its atypical course.

The second requirement concerns the destruction of lice (body and head lice) as carriers of TF infection as well as the delousing of the patients and the foci of infection---primary by the place of the patients' residence, and secondary, somehow connected with the primary foci according to the data of epidemiological analysis.

Here, TF patients and to an equal degree also all categories of patients suspected of TF, are subjected to a sanitary treatment at the site of their hospitalization (or special isolation), and the inhabitants of the foci are subjected to such treatment in the sanitary screening stations and other establishments for sanitary treatment. All kinds of lousy or suspected clothes and bed linens which belong to the patients and which come from the foci are subjected to treatment with modern means of disinsectization. The measures of disinsectization are also carried out in the corresponding premises.

After the sanitary treatment, all healthy persons coming from the primary and secondary foci of TF infection are subjected to a sanitary epidemiological observation for a period of two months, with measurement of their temperature for a period of 25-30 days. Moreover, all febrile persons are hospitalized, and in the hospitals they are investigated for TF by laboratory methods. In addition to thermometry, it is recommended to submit the observed persons to a second serological examination for TF infection, with the CBR test and the specific hemagglutination test at an interval of 10-15 days. Healthy persons who give positive serological reactions (subclinical forms?) are placed under special supervision with a second serological examination, and in case of necessity, they are preventively hospitalized.

In presence of epidemiological or special indices, in addition to the general measures of preventive sanitation of the population (see below), specific preventive measures can be also provided for the threatened contingents by way of single inoculations of live vaccine, which is below discussed in detail in a special chapter.

As domestic experience and foreign data indicate, the above mentioned measures will absolutely lead to the elimination of epidemic (louse-borne) TF.

In regard to sporadic, or second, forms of TF, the priority belongs to measures of a preventive order which start from the basic premises:---in presence of pediculosis, each case of sporadic (or second) TF is a potentially threatening source for the origin of epidemic forms of TF, which in their turn can create second (recurrent) ailments. Hence, the preventive measures of sporadic TF mostly coincide with the measures of control and prevention of epidemic TF.

Among these measures the possibly easiest detection of patients who have sporadic TF stands on the first place for their hospitalization. In its turn this task requires on the part of the clinician physicians still greater attention than in epidemic TF. If as we have already seen in epidemic TF, together with the predominant typical forms, there are atypical forms which present a certain difficulty for the differential diagnosis of their nature, which to a certain extent is facilitated by the epidemiological setup, then in sporadic TF, as we know, the sickness cases generally carry a mild character with considerable polymorphism of atypical forms, frequently running under different diagnoses such as grippe, pneumonia, catarrh of the upper respiratory pathways, paratyphoid, and so on. This peculiarity of ailments and the lack of an epidemiology characteristic for epidemic TF which makes physicians watchful, is complicated to a still greater degree by the detection of sporadic TF which very often, and at times in a predominant number of cases, is diagnosed only with the aid of serodiagnosis. But, experience

shows that, inspite of this, proper information of the physicians on the nature of the peculiar features of sporadic TF compensated the difficulty to a certain degree, even though by needless suspiciousness. Thus, according to the findings of Leningrad authors, up to 60% of the patients who were sent to the hospital under suspicion of sporadic TF, were discharged after the findings of serodiagnosis. This fact shows what an attention can be provoked to the danger of sporadic TF forms among the ranks of physicians. Thus, preventive hospitalization as a method of segregating TF in its mild and atypical forms in case of sporadic TF assumes a particularly important meaning.

The discussed material in itself exclusively predetermines the significance of serodiagnostic methods for the differential diagnosis of sporadic TF forms.

As to delousing as a rather important means in the control and prevention of TF, in its sporadic forms this problem also requires a special approach in view of its special epidemiological features.

It is well understood that, in the presence of lice in the patient or in his environment, measures of delousing must be carried out, as this is described for epidemic TF, including the same system of observance by contact persons.

But, as it is well known, in case of sporadic TF, the louse factor is often or usually absent at a simultaneous difficulty of recognizing the sources of infection. In addition, in the absence of exogenous sources of infection, it is generally difficult to get oriented as to the possibility and place of origin of the sporadic forms of disease. THEREFORE, the control of pediculosis as regards sporadic TF develops into a problem of sanitation of the population as to pediculosis in general.

As we have seen, modern hospitalization and disinfection of patients in epidemic TF, together with sanitary treatment and surveillance of the primary and secondary foci of infection, should guarantee the elimination of local and more disseminated outbreaks of epidemic TF.

As to sporadic, or second TF, the indicated measures are completely suitable to detoxicate the pertinent patients as potential sources of louse-borne TF infection, but they do not do away with the appearance of other sporadic ailments related to recurrences of earlier passed TF, which, according to the data of a number of authors (especially Romanian authors) are seen approximately in 3% of the persons previously sick with TF.

Thus, we can only talk of a reduction of the number of patients of sporadic TF, as the heritage of its epidemic forms, by way of radical measures of control and prevention of the latter. For it is completely evident that the number of sporadic TF ailments will be proportionate to the preceding number of sickness cases of epidemic TF. But means for the prevention of recurrences in the previously sick persons with TF are not known. It is true, since the recurrences are related to a loss or attenuation of the resistance of organism, it should be thought that the raising of the living standard in the sense of nutrition, hygiene of life and so on should limit also the recurrences which would mean a limitation of the number of sporadic forms. It is possible that, even with immunization of the threatened contingents, e.g., with live vaccine, recurrences can be reduced or prevented. But this question has only a theoretical interest, and it cannot be subjected to study, which in our opinion could be appropriately carried out by vaccinating the corresponding age categories, especially those who are seropositive or who give allergic reaction with an antigen made from RPr. Finally, the possibility is not excluded that an energetic cure of TF patients with antibiotics will promote the sterilization of patients in respect to the pathogenic agent, and thus, will prevent the possibility of recurrences.

### INOCULATIVE PREVENTION OF TYPHUS FEVER

Modern inoculative prevention of TF is based upon the immunization of people with different types of vaccines made from *R. prowazeki*. Its history cannot be separated from the general history of the origin and development of the methods of accumulation and cultivation of rickettsiae under laboratory conditions, and as a whole it is determined by the success of this section of laboratory mastery and study of rickettsiae.

At present, the use of two types of vaccine made of *R. prowazeki* can be discussed:---the killed vaccine, and the attenuated live vaccine.

#### KILLED PROTECTIVE VACCINES.

Having established, together with S. PROWAZEK, the multiplication of TF rickettsiae in the stomachs of body lice, G. DA ROCHA LIMA was the first in 1917-1918 to use in an experiment infected lice as material for the preparation of TF vaccine from killed rickettsiae.

Having used a phenolized mixture made of body lice, infected by feeding, he became convinced in guinea pig experiments that three inoculations of this vaccine in doses containing 5, 10 and 20 lice usually cause an immunity in these animals to their further infection with TF virus (DA ROCHA LIMA, 1918). The indicated experiments of DA ROCHA LIMA were also the starting point for all subsequent works on the preparation of preventive TF vaccine of different types made from killed Rickettsiae *prowazeki*.

#### I. LOUSE VACCINE (VACCINE OF WEIGL, AND VACCINE OF PSHENICHNOV-RAIKHER).

The above quoted observations of DA ROCHA LIMA were limited only to experimental examination of the TF vaccine from rickettsiae of infected lice. Further improvement and practical use of the vaccine of this type was carried out a little later by the Polish investigator R. WEIGL who for the preparation of "louse" TF vaccine used a method which he elaborated for the accumulation of *R. prowazeki* in the gastro-intestinal tract of lice by inoculating them through a rectal microenema. Using this method for the preparation of vaccine, WEIGL employed the intestine of infected lice which is filled with rickettsiae, by triturating them in a 0.5% solution of phenol. The author studied in detail the preventive effect of this vaccine on various animals (guinea pigs, rabbits, monkeys), and further on he conducted the first immunization of people with this vaccine, having determined simultaneously the accurate dosage of the vaccine (WEIGL, 1921-1930).

This is how the generally known "WEIGL vaccine" was created, and further on it was slightly modified with the addition their fecalia to the intestines of infected lice at the rate of 1:1.5 (CHRZANOWSKI, and G.S. MOSING, 1931).

The WEIGL vaccine was rather widely used in Poland (CHODSKO, 1933), in China among the missionaries (RUTTEN, 1936), as well as for individual prevention of laboratory workers (The L'vov and the Tunis institutes and others). As a result it was detected that this vaccine, at its full dosage, does not protect from TF disease, but it makes its course easier in case of infection, and it leads to a zero mortality.

According to the original prescriptions of WEIGL (1930), a triple inoculation with his vaccine was considered in doses corresponding to 25, 50 and 100 intestines of infected lice, which in sum amounted to about 9 billion rickettsiae. In the years of the Second World War, WEIGL sharply reduced the dosage of his vaccine to 25-30 intestines per an inoculation course. In Poland, about a million persons were inoculated by this method; moreover, according to the excessively optimistic statement of the author, the results of the inoculations could "surpass the expectations" in spite of the reduced dosage.

In the years of the Second World War, A.V. PSHENICHNOV and B.I. RAIKHER used a formalinized (0.2%) suspension for vaccine made of triturated larvae of lice which were infected with *R. prowazeki* by feeding them through an epidermomembrane according to A.V. PSHENICHNOV, and freed from chitin particles by centrifugation. This vaccine is prepared at the rate of 100 larvae per 2.5 ml of physiological saline, and in the indicate volume it contains about 3-4 billion rickettsiae which are introduced with triple inoculations in doses of 0.2 - 0.8 - 1.5 ml. (A.V. PSHENICHNOV, 1943, B.I. RAIKHER, 1943; A.V. PSHENICHNOV, B.I. RAIKHER, E.G. NOSKOVA, 1945). As it was proved, in people with a dosage of 50-100 larvae and in a case of a massive contact the effect of the vaccine is not large, but in case of a dosage of 100-200 larvae, and in the presence of a weak contact the vaccine displays high protective properties (A.V. PSHENICHNOV, 1944).

The PSHENICHNOV-RAIKHER vaccine, differing from the WEIGL vaccine by the simplicity of mass preparation, found a rather wide employment in our country during the Second World War.

At the present time, the WEIGL vaccine, just as also the PSHENICHNOV-RAIKHER vaccine, have essentially a historical significance in presence of other more rational types of vaccine (See below).

## II. EGG VACCINE (THE VACCINE OF COX).

The egg vaccine of COX is prepared from *R. prowazeki* which are accumulated in the vitelline sacs of chick embryos by the method of COX. At the preparation of this vaccine a suspension of rickettsial vitelline sacs is killed with formalin, and then from it the rickettsiae are extracted by way of ether treatment according to the method of CRAIGIE (1945). This rickettsial suspension, which is purified from the admixture of the vitelline sac and is killed with formalin in a certain concentration to which a preservative agent is added, is also the so-called egg TF vaccine of COX which is standardized as to its capacity to produce antitoxic immunity in guinea pigs or in mice.

During the Second World War the egg vaccine of COX had a very wide use in the USA and in England for the immunization of military units. It was also rather widely used in certain districts especially threatened in epidemiological relation for troops, and among the local civil population (EGYPT, Iran, Iraq, and so on).

Among the Americans, the egg vaccine of a definite standard was used three times in a dose of 1 ml, with subsequent revaccination 6 months thereafter, or two inoculations in the same dosage, but with revaccination at the start and in the middle of the epidemic season.

### III. PULMONARY VACCINE (DURAND-GIROUD VACCINE).

The pulmonary TF vaccine is prepared from *R. prowazeki* which were accumulated in the lungs of animals after intranasal or intratracheal infection. The best percentage of the pulmonary vaccine results in white mice which, with a certain procedure and regime, can provide vaccine production at very large scales (M.K. KRONTOVSKAYA and collaborators, M.M. MAEVSKII and collaborators).

From the pulmonary mass, the formalin-killed rickettsiae are extracted either by way of differential centrifugation (vaccine of the DURAND-GIROUD type), or else with the aid of ether treatment (ether-pulmonary vaccine of M.M. MAEVSKII).

In the years of the Second World War, the pulmonary vaccine, only partly used abroad (mostly in France), was the main inoculative preparation against TF in our country in the Army, and among the threatened civil population.

In the war years, the vaccine was used at a relatively low concentration (approximately 250 billion in 1 ml by bacterial standard, with a triple administration of the vaccine in doses of 0.5 --1 --1 ml at 5-7 day intervals).

In the postwar period, the concentration of the vaccine was increased 2-4 times, and, moreover, they started to release a lyophilic dry vaccine, deposited with calcium phosphate (M. KRONTOVSKAYA and collaborators). It should be mentioned however, at the same time that the latter type of depot vaccine proved to be highly reaction-provoking.

### EFFICIENCY OF THE KILLED VACCINES.

Comparison of the data which accumulated in the literature on the efficiency of various vaccine types, louse, egg, and pulmonary vaccines, shows that all types of the pertinent vaccines made from killed RPr. display mainly identical protective action. As experimental and clinical epidemiological observations show, the level of efficiency of killed TF vaccines can considerably vary, but these variations are conditioned not by the vaccine type. They are conditioned by other factors, for instance, the rickettsial content in the vaccine and their dosage, the form of vaccine (the ordinary form or the depot form), and the manner of their employment (repetition of inoculations, combination of primary immunization and revaccination).

As to the efficiency of killed vaccines made of RPr, according to the general acknowledgement of both domestic authors (A.F. BILIBIN, N.N. ZHUKOV-VEREZHNIIKOV with collaborators, M.K. KRONTOVSKAYA, A.A. GRINFELD et al., D.S. SHASTNYI and N.YA. EREMEEVA, S.D. GLADKIKH, A.B. ALEKSANYAN, K.F. KATSITADZE, E.M. MESHASHVILI, S. FAIFSTEIN and K. BEZDENEZHDIKH and others, 1944-1949), and foreign investigators (J. SADUSK, 1947; S. ECKE et al, 1945; STEWART-CARRIS et al, 1945; DURAND, 1943-1944; SACHS, 1946 and others), inoculations against TF with the aid of killed vaccines do not guarantee the inoculees against TF infection, but they regularly convert TF into a benign ailment.

As an illustration of the antiepidemic effect of the quoted inoculations, here are the below quoted summarized data which were published by Soviet authors during 1945-1949 and which mostly refer to the period of the Second World War. According to these data, among 43,034 inoculees, 133

sickness cases were recorded, or 30.9 per 10,000, while during the same time among 67,488 non-inoculated persons, 446 sickness cases were observed, or 77.5 per 10,000. In other words, among inoculees sickness cases occurred as an average  $2\frac{1}{2}$  times less often compared with non-inoculated persons. It should be added to this that among the whole bulk of inoculees and sick persons, only two cases had a fatal outcome, while among the non-inoculated persons the TF mortality was observed in the range of from 4.2 - 5.9 to 10.7 - 12.5% (P.F. ZDRODOVSKII).

Yet, it should be considered that the quoted statistics characterize the average efficiency of inoculations which can considerably vary in individual groups of inoculees depending upon the seriousness of epidemics and the general conditions.

In inoculees and sick patients, as it was already remarked, TF has a mild course, which is in a not always recognizable form. Hence the question arises, to what extent these light forms are infectious for lice, which is the same as the question about the epidemiological importance of light TF among inoculees which sometimes appear in obliterated forms. The findings available in the literature on this question are as follows.

In cases of serious course of TF among the inoculees, which is infrequently observed, on these patients the infection of lice can happen in the usual order (EKKE and others, 1945). As to the light forms of TF in inoculees, in these cases the infection is also possible, yet only to a limited or very limited degree.

Thus, according to the data of V.I. MITROFANOVA (1945), who observed 15 TF patients among the inoculees, lice were infected in 2.5% - 20% of the atypical forms, and in 20%-39% of the light forms of the disease.

Together with this, according to the observations of SNYDER, MURRAY et al (1949), with long-lasting (10 days) feeding of lice on TF patients, the intensity of lice infection is 230 times less among inoculees, compared with non-inoculated patients.

In conclusion it is not without interest to point out the determination of the efficiency of inoculations with killed vaccine which were made lately by American authors under experimental conditions on volunteers.

Thus, out of 6 double immunized persons with the COX vaccine, at their infection with TF virus three got as sick as the controls, two had the sickness in a mild form, and one did not get sick. At the same time, among 6 primarily immunized and revaccinated volunteers, at their subsequent infection, one got sick, while 5 persons showed complete resistance (FOX et al, 1959).

The quoted experiments clearly show a perfectly unsatisfactory efficiency of the primary immunization, and, in contrast, a high efficiency of the primary immunization in combination with revaccination which should be thus considered as an obligatory measure at the employment of killed vaccines against TF.

#### PROBLEMS OF LIVE VACCINE.

As we have seen, inoculations with killed vaccine made of RPr can be fully efficient under the condition of sufficient concentration of rickettsiae in the inoculative preparation, and with the use of double immunization in obligatory combination with a later revaccination.

Nevertheless, the inoculative prevention of TF with the aid of killed vaccine has practically no perspective, or only a perspective in narrow limits. This is caused by two circumstances:---the methodical complexity of the required second inoculation and the difficulty which makes unreal the preparation of a vaccine of the necessary concentration at a large scale of inoculations. Moreover, in inoculations with killed vaccine, the production of immunity requires considerable time. All in all, as a rational measure of planned prevention of more or less limited, organized contingents, the immunization with killed vaccine cannot be used as an antiepidemic means.

By analyzing the problem of killed vaccine in its final review, the greatest American specialists come to the similar conclusion, emphasizing that only the live vaccine can satisfy the requirements of antiepidemic prevention of TF under emergency conditions (E. SMADEL, B. JACKSON, M. CAMPBELL, 1959).

The advantage of live vaccine as an antiepidemic means against TF is entirely obvious:---the live vaccine is easily prepared with a minimum of losses and it can be stored in an unlimited quantity. It is simple in use, since it is used in a single inoculation. Finally, the live vaccine quickly produces immunity, and thus it can counterattack an epidemic.

Hence, already previously the investigators, especially according to the School of CH. NICOLLE (Tunis), paid attention to the search of a live vaccine against TF. During 1934-1940, the French authors, at their head with CH. NICOLLE, had arranged to a certain extent a preparatory stage for the solution of the problem of live vaccine with the use of "attenuated" strains of rat rickettsias as a live vaccine against epidemic TF, whose pathogenic agent is close to R. mooseri according to its antigenic structure. CH. NICOLLE and LEGRE (1936) proposed for this purpose the brain virus of rat rickettsias (The E strain of SPARROW), attenuated by exsiccation in a phosphate buffer salt mixture, and then made into an emulsion in chick vitelline membrane (encasing of the vaccine) with sufficient emulsification in a vegetative oil (additional encasing).

BLANC, the pupil of CH. NICOLLE, used treatment with bile for the attenuation of the virus of rat rickettsias. His "bilivaccine" was originally (1934-1938) prepared from a suspension of organs of infected guinea pigs (suprarenals, spleen, testicular membranes), which were treated with bile, and later on (since 1938) the author prepared the "bilivaccine" from "fecal virus" treated with bile, and taken from fleas which were infected on rickettsial rats.

With the indicated vaccines and particularly with the "bilivaccine" of BLANC, in North Africa mass inoculations of the population were undertaken against epidemic TF. Suffice it to say that during 1934-1940 more than 2 million persons were inoculated with the "bilivaccine" of BLANC.

In the opinion of the authors, the vaccines from Mooser's rickettsiae in their above given samples proved to be completely tolerated and highly effective so that in a number of cases, for instance, with the aid of BLANC's "bilivaccine" the epidemic could be cut short (BLANC, 1937; BLANC and BALTHASAR, 1941).

However, the simultaneous and subsequent researches of BLANC's "bilivaccine" in Chile (1935) and in Japan (1944) showed the dangerousness of this vaccine which not infrequently caused sickness cases of rat rickettsias among the inoculees, at which several persons among the sick died (F. PALACIOS, R. CHAVEZ, O. AVEDANO, 1935; SADUSK and H. KUHNENBECK, 1946).



Thus, the French investigators, having established the unquestionable prospects of a live vaccine, could not offer yet a reliable preparation of the latter.

In 1943, this question reached its definite solution in the lucky discovery of the Spanish investigators CLAVERO DEL CAMPO and PEREZ GALLIARDO, who became the possessors (holders) of the E strain of RPr which lost virulence, spontaneously splitting off from the egg culture of the virulent "Madrid" strain in the 14th passage. Studied in a preliminary manner in Spain in animal experimentations, and tried out not without success in mass inoculations of people, in its 225th passage in chick embryos the E strain was transported to the USA where it was also submitted to detailed study by the collective of American authors under the leadership of Prof. FOX, with a wide trial of inoculations on people. In a summary, the mentioned observations permitted the American authors to explain with greater reliability the harmlessness and reactigenous properties of the live vaccine E, to work out its dosage for people, as well as to study its immunogenicity and efficiency in direct trials by infecting inoculated persons with TF virus. The main results of this extensive cycle of investigations could be briefly summarized in the following manner.

#### DATA OF AMERICAN AUTHORS ON LIVE VACCINE E.

For vaccine the American authors used an egg culture of rickettsiae of strain E in the form of a lyophilic exsiccated 10% suspension made of vitelline sacs of infected chick embryos, by determining the rickettsial concentration in the vaccine according to their content of infectious doses for chick embryo (MIDE). In a dry vaccine this concentration corresponded to  $10^{7.5}$  indicated doses.

The dry vaccine can be kept in refrigerator without change of standard for several months. Before use, the dry vaccine is dissolved in sterile distilled water and diluted to the required concentration in a sugar-phosphate-glutaminic buffer solution. The diluted vaccine is used in a dose of 0.25 ml subcutaneously and intramuscularly; moreover, it was tested for reactivity and immunogenicity in concentrations of 1, 0.25 and 0.1%. It was also detected that vaccine E causes early reactions soon after its administration and late reactions which appear 9-14 days afterwards and correspond to vaccinal TF infection. Below are given the data of American authors on the reactivity of the most tolerated and sufficiently immunogenic 1% concentration of the vaccine, as it appears in early (Table 27) and late (Table 28) reactions.

EARLY REACTIONS

Таблица 27

Ближайшие реакции

1 Число привитых и контроли	2 Реакция, %						
	3 лихорадка	4 головная боль	5 температура	6 проказа	7 общее недомогание	8 местная реакция	9 лимфаденит
955	5	17	2	1	8	7	7
10 Контроли 885	2	7	1	1	2	1	1
11 Итого с коррекцией	3	10	1	0	6	6	6

**TABLE 27.** - 1...number of inoculees and controls; 2...reaction in %; 3...fever; 4...headache; 5...nausea; 6...vomiting; 7...general malaise; 8...local reaction; 9...lymphadenitis; 10...control 885; 11...Total with correction.

LATE REACTIONS

1 Число привитых и контроли	2 Реакция, %							3 Характер реакции, %			
	4 все степени	5 по степеням			6 по длительности в днях			7 лихорадка	8 головная боль	9 болезненность, работа	10 постельный режим
		1	2	3	1-3	4-6	7+				
724	26	19	6	1	12	11	3	5	25	3	1
11 Контроли 885	11	9	2	0	7	3	1	2	11	1	0
12 Итого с коррекцией	15	10	4	1	5	8	2	3	14	2	1

**TABLE 28.** - 1...number of inoculees and controls; 2...reaction in %; 3...all degrees; 4...by degree; 5...by duration in days; 6...character of reaction in %; 7...fever; 8...headache; 9...nausea, vomiting; 10...bed regime; 11...control 885; 12...Total with correction.

Thus, in the next few days after inoculations (with correction for the control, marked direct reactions were associated in 3% with short fever; in the majority of cases (10%) they were restricted to headaches and general malaise (6%), in presence of local reaction and lymphadenitis in 6%.

As to the late reactions, i.e., symptoms of vaccinal infection, with correction for the controls, they were noted in all forms and degrees, in 15%, including a marked degree only in 1%, which made bed regime necessary, with a length of the reactions in the majority from 1-3 (5%) up to 4-6 (8%), rarely (2%) lasting 7 days or longer.

The late reactions were manifested chiefly in the form of headaches (14%), with presence of fever in 3% of the cases.

The immunogenic effect with its verification in 215 inoculees was manifested by a positive CBR of some degree in 89%, and in a combination of this with toxin neutralization in 95%.

Such are the main results by studying the reactigenicity and the immunogenicity of live vaccine E at its  $10^{-2}$  concentration, as they were reflected in a subsequent summary of American authors (J. FOX, J. MONTOYA, M. JORDAN et al, 1959).

For the illustrations of the protective efficiency of live vaccine E, the American investigators carried out experiments with infecting inoculee volunteers with a virulent strain of Rickettsia prowazeki (the BREINL strain).

In this experiment out of 42 inoculees with the E vaccine who were infected in a period of 2 months to  $5\frac{1}{2}$  years after the inoculation, in 37 persons the infection was without results; in 3 persons, obliterated forms of infection developed, and in 2 persons there was marked sickness which did not differ from the controls who accompanied the experiments in all times of the research on immunity in the inoculees (FOX and collaborators, 1954-1959). Thus, a well-manifested protective action of vaccine E was displayed in the experiment in regard to the virus of TF infection, with its maintenance in the inoculees not less than 5 years.

Finally, it should be mentioned that by the same data of American investigators, the persons inoculated with live vaccine E could not present any danger in an epidemiological respect, since there are no rickettsiae in them, and after the introduction of the vaccine, lice cannot become infected at feeding on the inoculees for 10 days' duration (quoted after FOX, and others, 1959).

#### PERSONAL OBSERVATIONS WITH THE "E" LIVE VACCINE.

At the end of 1955, due to the kindness of Prof. G. FOX, by the intermediation of Dr. K. FILIPP (Montana, USA), we obtained a strain E of RPr which in the subsequent years (1956-1962) was subjected first to detailed experimental study (1956-1957), and later on it was studied as a live vaccine for inoculation of people, at first for organized contingents, and later in a wide experiment of mass immunization (1960-1962).

The results of this large long-range experiment, which to a considerable degree corroborated the above outlined data of American investigators, can be summarized in a general outline as follows.

The experimental portion of the work in our laboratory established that strain E did not differ at all from the virulent strains of RPr in regard to morphology, cultivation in chick embryos, and serology. At the same time, it differs very much in its pathogenic properties in experiments on guinea pigs, in which even at a maximum dosage ( $10^{-1}$  to  $10^{-2}$  MID = minimum infective dose) at intraabdominal inoculation it causes only a benign symptomless infection, while the virulent BREINL strain under the same conditions of infection causes a febrile reaction in guinea pigs in doses of  $10^{-7}$  -  $10^{-8}$  (P.F. ZDRODOVSKII, E.M. GOLINEVICH, V.A. YABLONSKAYA). At the same time, the asymptomatic forms, which are caused by doses  $10^{-3}$  -  $10^{-6}$  of the E strain, showed complete resistance to intensive treatment of the infected guinea pigs with cortisone, while in case of their infection with the BREINL strain, even with a dose of  $10^{-8}$ , under the effect of cortisone,

marked activation of the asymptomatic forms was observed (V.A. GENIG).

The histopathological changes in guinea pigs infected with Strain E were also considerably less marked compared with pigs infected with the BREINL strain (I.N. KOKORIN).

As to the immunogenic properties of Strain E, they were manifested in it sufficiently well, even though somewhat weaker than in the virulent BREINL strain. Thus, doses of  $10^{-2}$  -  $10^{-4}$  of Strain E regularly caused a full or partial resistance in the guinea pigs to subsequent infection with the virulent BREINL strain. In doses of  $10^{-2}$  -  $10^{-6}$ , the effect of immunization varied, while in a dose of  $10^{-7}$  the immunity was absent.

At the same time, even in a dose of  $10^{-8}$  the BREINL strain regularly produced immunity in guinea pigs, with varying results for the doses  $10^{-9}$  -  $10^{-10}$ .

It is important to remark that in doses of  $10^{-2}$  -  $10^{-3}$  already on the second day after inoculation Strain E produced a resistance in guinea pigs to infection in the order of a promunition, while after a week the immunity level was equivalent to  $10^{-4}$  -  $10^{-5}$  infective doses of the BREINL strain (E.M. GOLINEVICH).

Such are the experimental characteristics of Strain E in their own outline, which permits us to treat it as a strain that corresponds to the experimental requirements raised against a live attenuated vaccine.

Having become convinced about the harmlessness and immunological efficiency (by CBR and HAR) of live vaccine E on a not very large group of volunteers, with the decision of the Committee of Vaccines and Sera at the USSR Ministry of Public Health, our laboratory carried out a mass vaccination test with the idea to have an objective opinion on the reactogenicity and immunogenicity of the vaccine in practical settings (V.A. YABLONSKAYA in collaboration with N.G. KENICHEVA, V.F. IGNATOVICH and G.M. DUTOVA).

For the immunization of people, E.M. GOLINEVICH prepared six series of lyophilic dried vaccine E which, at its standardization by infection of chick embryos with counting the presence or absence of rickettsiae in the smears with an incubation up to the 12th day inclusively gave the following results:---at  $10^{-7}$  dilution, rickettsiae were found in all six series in 14 out of 49 infected embryos, with variations from 2/9 to 4/9. At dilution  $10^{-8}$  the summary results were manifested at the rate of 2 positive findings for 46 infected embryos, with a variation of 1/8 to 0/9. Thus, the titre of the prepared and lyophilically exsiccated vaccine was equal to  $10^{-7}$  expressed in MIDE (= Minimum Infective Dose for chick Embryos (E.M. GOLINEVICH, 1962)).

The immunogenic properties of the prepared series of E vaccine were checked on guinea pigs by means of intraabdominal inoculation of the animals with 1 ml of the vaccine in a dilution of  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$ , with subsequent test of the immunity after 45-60 days by means of intraabdominal infection of the animals with a virulent BREINL strain (egg cultures) in a dose corresponding to 10,000 MID which in control guinea pigs causes a 5-7 day fever after a period of 3-5 days of incubation.

The summary data of this experiment in the dose of  $10^{-3}$  which especially interested for us were manifested in all six series in the following ratios:---out of 50 vaccinated pigs, 44 i.e., 88% displayed marked immunity,

including full immunity in 72% (in these pigs a febrile reaction was completely absent), and partial immunity in 6 (12%) (1-2 day elevation of temperature).

In all the vaccinated guinea pigs, the serum was tested at the same time. It gave positive CBR reaction in all cases with an average titre of 1:65.3, with variations for the individual series from 1:82.5 to 1:216.6 (E.M. GOLINEVICH).

For people the inoculative dose of the prepared live vaccine E, with the above indicated characteristics, in correspondence with the results of its standardization and the below quoted data of American authors, was found to be equal to 0.25 ml of a  $10^{-2}$  dilution, which corresponds to 4 ( $\sqrt{0.5}$ ) lg MIDE.

The vaccine of the indicated composition was poured into ampules, each getting 0.5 ml of rickettsial suspension in skim milk in a dilution of  $10^{-2}$  (by weight of the vitelline sacs) and lyophilically exsiccated, with subsequent soldering of the ampules under vacuum. When used, the dry vaccine in the ampuls is dissolved in 5 ml of sterile physiological solution, and used subcutaneously in a dose of 0.25 ml.

In a mass trial all together 2617 persons were simultaneously inoculated with this vaccine in an organized collective in the age groups from 16 to 55 years. Here, before the conduction of mass inoculations, 311 persons were immunized in advance. In this control group specially careful observations were made on the reactions to inoculation, as well as serological investigations were made with CBR and HAR before inoculations and 21-35 days after inoculations.

Let us go over to the evaluation of the reactigenicity of live vaccine E, first of all as it appeared in the control group of inoculees. Early non-specific reactions to the administration of the vaccine were observed in 191 persons of the control group.

Brief elevations of the temperature on the 2nd day after inoculation were recorded only in 3 people, i.e., in 1.6%. Local reactions in the form of hyperemia and slight infiltration on the site of inoculation were noted in 17 persons, i.e., in 9%. In general, the early reactions were manifested very weakly, and for the evaluation of reactigenicity of vaccine E they are without any interest.

Before discussing the late reactions, it should be mentioned that in the control group among 299 persons in one sub-group there were 147 persons in the age group from 16 years to 25 years among whom 99.7% (146 persons) was seronegative with CBR, and in a second sub-group of 152 persons in the age group up to 60 years 17% was sero-positive with CBR. In correspondence with this, considerable differences were observed in the reactions of the two sub-groups to vaccination. Thus, in the first sub-group of sero-negative subjects, 18 cases of late vaccinal reactions, or 12.2% occurred. In the second sub-group from the immune layer (17%), only 6 late reactions were recorded, or 4%. These reactions are given in detail

according to their intensity as follows (Table 29 and Figure 40)<sup>1)</sup>.

From Table 29, it is thus evident that the percentage of the manifestation of late reactions depends upon the immunological "background" of the inoculees. They are mostly manifested quantitatively and qualitatively in subjects who are sero-negative with CBR with a RPr antigen (12.2%), and to a much lesser degree in the presence of an immune layer in the collective. Specifically, with the 17% of sero-positive subjects, late reactions were noted only in 4%.

Таблица 29

1	Подгруппы	2 Количество прини- тых	3 Показание реакции				7 Всего	8 %
			4 слабая 37,2° 1 день	5 средняя 37,6-38,5° 3-4 дня	6 сильная 38,6° 5-7 дней			
8	I серонегативная	147	1	10	7	18	12,2	
9	II — 17% сероположительных	152	0	6	0	6	3,9	
10	Итого . . .	299	1	16	7	24	7,7	

TABLE 29. - 1...Sub-groups; 2...number of inoculees; 3...late reactions; 4...weak, 37.2°, 1 day; 5...medium, 37.6° - 38.5°, 3-4 days; 6...strong, 38.6°, 5-7 days; 7...total; 8...I. sero-negatives; 9...II sero-positives, 17%; 10...TOTAL

From the control group, 157 inoculees were serologically examined with CBR with HAR with a RPr antigen. The results of these examinations are given in Table 30.

Thus, from Table 30 it is evident that vaccine E causes a very good immunological shift ---in 97% for CBR, and in 95% for HAR; however, in 72% of the inoculees, HAR was found in high and very high titres. It should be especially emphasized that among 143 inoculees who did not give late reactions i.e., with an asymptomatic course of the vaccinal TF infection, a positive HAR in high and extremely high titres (from 1:1600 to 1:51200) was observed in 50.3%.

FOOTNOTE 1): The late reactions as they are shown in Figure 40, while producing different individual responses to the vaccinal infection, at the same time illustrate the different resistance or susceptibility to TF in those who make the collective. It is perfectly obvious that people who have marked late reaction to the inoculation with live vaccine (see graph a,b), are particularly sensitive and threatened by TF, in difference from people who react to live vaccine with an asymptomatic infection with strong immunological indices (see graphs d and e). Thus, inoculations with live vaccine not only reveal the non-homogenous susceptibility of the collective to TF, but they also reveal people who are mostly threatened by this infection. At the same time, the indicated finding explains the inevitable diversity of clinical manifestations of TF infection in people who were subjected to infection as a reflection of their different sensitiveness to the pathogenic agent, which is actually the case (see page 53).

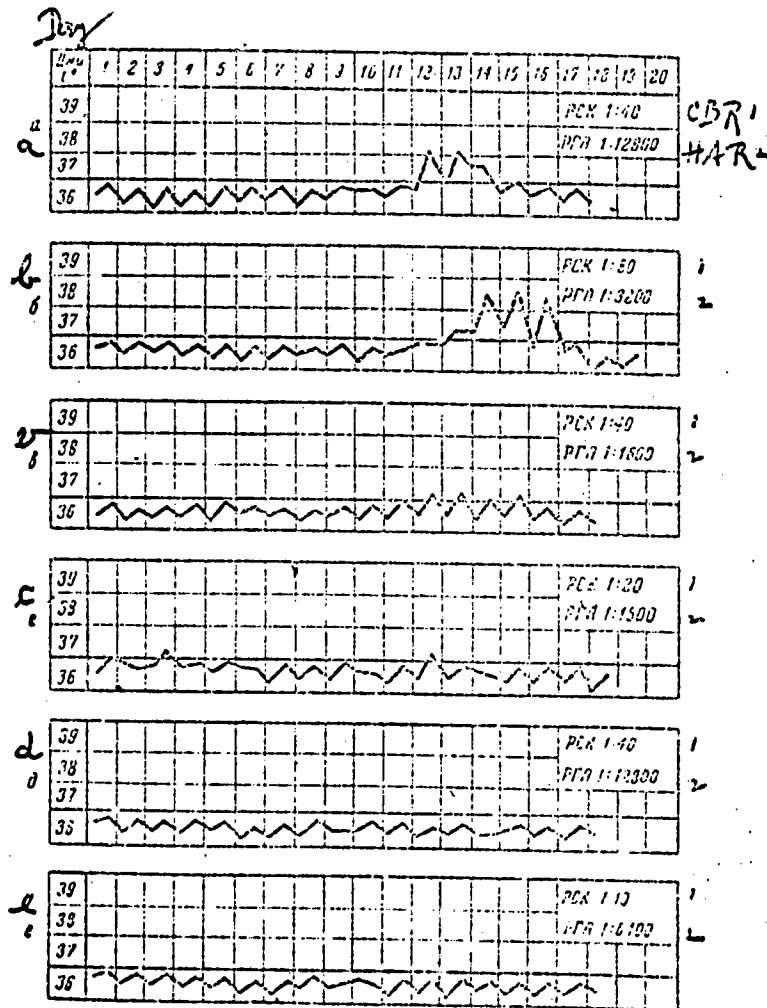


Рис. 40. Типы температурных кривых и серологических ответов по РСК и РГА у интактных живых вакцин из штамма Е риккетсий Провачека (по Е. М. Голынец и В. А. Яблонская, 1961).

а, б — выраженные поздне реакции; в, с — слабо выраженные поздне реакции; д, е — бессимптомная вакцинальная инфекция.

FIGURE 40. - Types of fever graphs and serological responses with CBR and HAR in inoculees with live vaccine from the E strain of *Rickettsia prowazekii* (after E.M. GOLINEVICH and V.A. YABLONSKAYA, 1961).  
a, b...marked late reactions; v, c...weakly marked late reactions; d, e...asymptomatic vaccinal infection.

Таблица 30

1 Число обследо- ванных по РСК	2. Титры по РСК						
	0	1:10	1:20	1:40	1:80	1:160	1:320
157	5	3	37	38	36	8	5

3 Число обследо- ванных по РГА	4. Титры по РГА									
	0	1:100	1:200	1:400	1:800	1:1600	1:3200	1:6400	1:12800	1:25600
157	8	4	11	21	31	23	24	19	8	9

TABLE 30. - 1...Number of persons examined by CBR;  
2...Titres for CBR;  
3...Number of persons examined by HAR;  
4...Titres for HAR.

Let us now pass over to the analysis of the reactigenicity of live vaccine E, as it was revealed in the mass inoculation practice. The early reactions did not cause any complaints, and they were not taken into consideration, since they are not much characteristic for vaccination in inoculees at mass inoculation. As to the most important late reactions, they are considered on the basis of the data from the medical posts which served the inoculees, where the latter were also returned with any kind of complaint.

Late reactions of different intensity were noted in 115 persons among 2335 inoculees, i.e., in 4.9% of the inoculees; moreover, in regard to the inoculations 92 medical certificates were issued which included about 4% of the total number of inoculees.

Late reactions occurred 10 to 19 days after the inoculations. They usually started with chill and increase in the temperature, being accompanied by headache and myalgia, and general malaise. In 12 inoculees, on the skin of the abdomen and the lateral surfaces of the arms, an eruption was noted in the form of isolated roseolae, which disappeared after 12-72 hours.

Таблица 31

1 Число привитых	2 Число привитых с поздней реакцией	3 Число выданных больничных листов	4. Характеристика поздних реакций по максимальной температуре			
			5. Слабая до 37,5°	6. Средняя 37,6-39,5°	7. Сильная 39,6-39,9°	
2335	115	4,9	92 (4%)	27 (1,1%)	63 (2,7%)	22 (0,9%)

TABLE 31 - 1...number of inoculees; 2...number of inoculees with late reactions; 3...number of issued medical certificates; 4...characteristics of late reactions by maximum temperature; 5...weak; 6...moderate; 7...marked.



The late reactions are characterized in more detail by the summarized data of Table 31.

Table 32 gives the duration of late reactions in the inoculees.

Таблица 32

1 Число учтенных привитых	2 Длительность лихорадочной реакции в днях							
	2	3	4	5	6	7	8	9
100	16	18	28	21	11	4	1	1
		83%				17%		

TABLE 32. - 1...number of considered inoculees;  
2...duration of the febrile period in days.

In the inoculees with late reactions no complications were observed. There was no convalescent period.

Since the inoculations were made in a large collective simultaneously in several cities, in the evaluation of late, especially serious reactions we should also count with the possibility of associated infections and sicknesses, as this was also made by the American investigators. In our experiment such controls were not made for the correction of reactions. Therefore, the possibility is not excluded that there is a certain overstatement in the indices of reactigenicity.

Thus, in full correspondence with the of American authors, for the overwhelming majority of inoculees, the live vaccine E is shown fully tolerated and are active. At the same time, approximately in 10%-12% (against 15% accounting to American authors) in sero-negative subjects, especially who are susceptible to TF infection, some kind of manifestations of a late febrile reaction is observed. In the presence of an immune layer, which is regularly met with in the adult population of localities which in the past were afflicted to some extent with TF, the number of late reactions among the inoculees is lower, evidently in proportion to the manifestation of the immune layer. At the present time, with the presence of 17% positive CBR in selectively examined groups, the percentage of late reactions dropped approximately twice (5) compared with the sero-negative contingent (10-12).

But under all conditions, late reactions are clinical manifestation of a vaccinal TF infection in persons who are particularly predisposed to it, and, in spite of their fully benign course, they are an essential shortcoming of live vaccine E, the more so, since according to both our and the American observations, in 1% of the cases, the late reactions force people to bed confinement.

In correspondence with the indicated, the elaboration of a methodology for the estimation of late reactions is extremely important. It is a problem which is within the reach of an easy solution as the observations in our laboratory showed. By combining a certain dose of solved antigen of RPr with the live vaccine E which during the incubation period (9-10 days) of late reactions will cause a slight resistance that suffices to prevent

the clinical manifestation of a vaccinal infection in the form of late reactions (V.A. YABLONSKAYA, 1963).<sup>1)</sup>

In a final summary, live vaccine E can be characterized as at present an unique reserve antiepidemic means to be used for stopping T<sub>h</sub> epidemics under menacing conditions with the aid of specific inoculations.<sup>2)</sup>

Inoculations with live vaccine E do not mean any kind of epidemiological danger, since---in correspondence with the observations of American authors and according to the data of domestic investigators---lice cannot be infected when they suck the blood of inoculees even at the height of the strongest late reactions (V.A. PSHENICHNOV, A.A. LEVASHEV, V. YA. NIKOLENKO, 1959), and at the repeated passages on infected lice Strain E does not change its properties, i.e., it remains non-pathogenic (V.A. PSHENICHNOV, et al, 1959).

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FOOTNOTE 1); Lately, in a massive inoculative experiment it was found that the combined live vaccine E, i.e., the original vaccine E in association with the solved antigen of RPr, is a very immunogenic and well-tolerated inoculative preparation.

Thus, at the immunization of 1380 persons, with the combined vaccine E, who were 80% in the age group from 16 to 30 years, and were sero-negative by CBR with a Promazek antigen in 95%---late reactions were recorded only in 65 persons, i.e., in the range of 2%; moreover, all reactions were absolutely mild (V.A. YABLONSKAYA, 1963).

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FOOTNOTE 2); The "combined live vaccine E" modified in our laboratory, i.e., the original vaccine E in combination with the solved antigen from RPr, obtained in 1964, the official approval of the USSR Ministry of Public Health after its approval by the Committee of Vaccines and Sera.

SUPPLEMENTMETHODS OF SETTING UP SEROLOGICAL REACTIONS IN TYPHUS FEVER (1)

(1) Compiled by E.M. GOLINEVICH, Dr. med. Sciences.

For the diagnosis of typhus fever, the below listed serological reactions are used with antigens made from *Rickettsiae prowazeki*.

1. The complement binding reaction (CBR).
2. The hemagglutination reaction (HAR).
3. The rickettsiae agglutination reaction (RAR).

THE COMPLEMENT BINDING REACTION1. Condition of making the complement binding reaction.

For setting up the complement binding reaction (CBR), five ingredients are needed:

- 1) the tested serum;
- 2) the antigen;
- 3) the complement;
- 4) the hemolytic serum;
- 5) sheep red cells.

The reaction is made in a total volume of 1.25 ml, i.e., 0.25 ml of each ingredient. For saving antigen, the reaction can be also set up in a volume of 1 ml, i.e., 0.2 ml of each ingredient.

The first phase of the reaction, i.e., the binding of the complement, can be carried out in the cold at 4° in 18-20 hours, or at 37° temperature in one hour. The second phase of the reaction is carried out in both cases at 37° in 30 minutes. Preferably the "cold" method is used for the binding, since in a number of cases the reaction is rendered more sensitive.

An obligatory condition for setting up CBR is the use of accurately titrated ingredients. The complement is titrated every time on the day of setting up the main test, regardless whether it is used in its fresh or preserved form. The antigen and the hemolytic serum are titrated by those institutes which manufacture them, and, in correspondence with the given titrations, on the label of the dry antigen the volume of liquid is indicated in which it should be dissolved, and on the label of the hemolytic serum its titre is stated.

For the sake of accuracy of the obtained results and the continuity of tests, each time parallel with the tested sera the reaction must be also set up with a control of the specific immune serum (with a definitely positive, known titre).

For the reaction of complement binding, separate vessels must be used (pipets, test tubes, flasks), which are thoroughly washed, without the use of caustic lye or acids. The washed vessels should be dried in a dry-heat box.

For each of the reaction ingredients (sera, antigen, complement, hemolytic sera, red cells) separate pipets are used.

All dilutions are made with sterile physiological solution (0.85% solution of chemically pure table salt in distilled water).

## 2. PREPARATION OF THE INGREDIENTS OF THE COMPLEMENT BINDING REACTION

1) The serum. - For getting serum, blood is taken from the vein or from the finger (not less than 1.5 ml).

The serum to be tested should be separated from the blood clot, and transferred into a clean tube without red cells (if necessary, it should be centrifuged).

The sera must be inactivated, i.e., heated in a water bath of 56° for 30 minutes. Attention should be paid to it that the sera placed for heating in the water bath should not touch the bottom which could mean an excessive heating of the serum, and also its coagulation.

The sera which are to be tested in the CBR can be further (for months) preserved also in the liquid form, without change of their titres provided that they are kept in a refrigerator with the prevention of their exsiccation and moulding. For the preservation of liquid serum, successfully boric acid can be used which is added in the amount of 1-2%. Sera which are strongly hemolyzed or contain red cells can be anti-complementary, i.e., they can inhibit hemolysis also in absence of a specific antigen. Such sera are not suitable for the reaction.

2) The antigen. - For the serodiagnosis of TF with CBR, an antigen is used made from *Rickettsiae prowazeki*. This antigen can be either a well-purified suspension of rickettsiae in 5% saccharose, dried out in vacuum (corpuscular antigen), or it is in the shape of a so-called dry whole antigen which consists of partially dissolved rickettsiae cultivated in the vitelline membranes of chick embryos.

On the label of the ampul containing dry antigen it should be indicated with which volume of physiological solution it should be dissolved so that it should correspond to a content of not less than 2-4 antigenic units in a 0.25 ml volume (or 0.2 ml antigen if the reaction is conducted in a volume of 1 ml).

The dry antigen is dissolved in physiological saline before the arrangement of the reaction. The dissolved liquid antigen can be kept for some time in the refrigerator, and used if there is no bacterial contamination.

The ready antigen need not to be titrated. However, in the process of work there may be need to check the quality of the antigen. In such cases, the antigen is verified in a titration test according to the following outline.

The dry antigen is dissolved in the volume of physiological saline which is shown on the label of the ampul, and it is diluted in a separate set of test tubes 1:2, 1:4, 1:8, 1:16, 1:32. Then, in three sets of test tubes (6 tubes for each set), 0.25 ml is poured from the antigen dilutions, (into the first tube 0.25 ml of the dissolved but undiluted antigen is poured). Further on, in each test tube of the first set with different dilutions of antigen, 0.25 ml specific immune serum is added (standard, sent together with the antigen) in a dilution corresponding to 4 times of its titre. For instance, if the serum titre is 1:320, then for the titration of the antigen a dilution of 1:80 must be taken, i.e., the ampul with 0.1 ml dry serum is diluted with 8 ml of physiological saline.

In the second set of test tubes with different dilutions of antigen, 0.25 ml non-specific immune serum is added to each tube in a dilution of 1:20. e.g., at the titration of the RPr antigen we can take a serum against Burnet rickettsiae or D. sibericus as the non-specific immune serum.

In the third set of test tubes with different dilutions of antigen, 0.25 ml physiological saline is added to each.

Parallel with this, controls are set up for the sera which were taken for the test (specific and non-specific sera) for testing their anti-complementary character:---in two separate test tubes 0.25 ml is poured from one and the other serum (of the same dilution which was used for the test), and, instead of the antigen, 0.25 ml physiological saline is added.

Then, to all test tubes 0.25 ml complement is added in a dilution which corresponds to the previously given titrations (see below), and a control is set up for the complement (0.25 ml complement in a dose taken in the test, / 0.25 ml physiological saline solution). The test tubes are shaken and put in a refrigerator for 18-20 hours, if the binding is entirely conducted in the cold, or in a thermostat at 37° for an hour. After the indicated time, the hemolytic system is added (see below), and the test tubes are put in the thermostat at 37° for 20-30 minutes until the completion of hemolysis in the controls. The reading of the results is done one hour after the tubes were taken out from the thermostat.

In Table 33 we give an outline of titration for the antigen which makes possible to find out how many antigenic units are contained in a given antigen, its specificity, and the presence or absence of anti-complementary properties.

## OUTLINE OF ANTIGEN TITRATION

Таблица 33

Схема титрования антигена

a	b	c № пробирки и разведение антигена						e Контроль			
		d						сыво-ротки	ком-племен-та		
		1	2	3	4	5	6				
раз-проби-рок	Интреанест	исход-ный	1:2	1:4	1:8	1:16	1:32				
A	1-й	1 Антиген	0,25	0,25	0,25	0,25	0,25	0,25	—	5 На 18—20 часов при 4° или при 37° на 1 час	
		2 Сыворотка специфиче-ская	0,25	0,25	0,25	0,25	0,25	0,25	0,25		—
		3 Комплемент	0,25	0,25	0,25	0,25	0,25	0,25	0,25		0,25
		4 Физиологический рас-твор	—	—	—	—	—	—	0,25		0,5
B	2-й	1 Антиген	0,25	0,25	0,25	0,25	0,25	0,25	—	6 Добавление гемолити-ческой системы по 0,5 мл	
		2 Сыворотка неспецифи-ческая	0,25	0,25	0,25	0,25	0,25	0,25	0,25		—
		3 Комплемент	0,25	0,25	0,25	0,25	0,25	0,25	0,25		—
		4 Физиологический рас-твор	—	—	—	—	—	—	0,25		—
C	3-й	1 Антиген	0,25	0,25	0,25	0,25	0,25	0,25	—	7 В термостат при 37° на 30 минут	
		2 Физиологический рас-твор	0,25	0,25	0,25	0,25	0,25	0,25	—		—
		3 Комплемент	0,25	0,25	0,25	0,25	0,25	0,25	—		—

TABLE 33. - a...Set of test tubes; b...ingredients; c...number of tube and dilution of antigen; d...initial; e...Control; f...serum; g...complement; A...First set; B...Second set; C...Third set. 1...antigen; 2...specific serum; 3...complement; 4...physiological solution; 5...At 4° for 18-20 hours or at 37° for one hour; 6...Addition of hemolytic system, 0.25 ml to each; 7...In thermostat at 37° for 30 minutes.

## EXAMPLE OF RESULTS OF ANTIGEN TITRATION

Таблица 34

Пример результатов титрования антигена

1 Антиген	2 Иммуная сыворотка риккетсий	3 Разведения антигена						4 Контроль	
		1:0	1:2	1:4	1:8	1:16	1:32	сыво-ротки	компле-мента
7 р. Проваче-ка сер. №	3 Провачека	4+	4+	3+	1+	—	—	—	—
9 То же	10 Бернета	—	—	—	—	—	—	—	—
11 " "	11 Без сыво-ротки	—	—	—	—	—	—	—	—

TABLE 34. - 1...Antigen; 2...Immune serum for rickettsiae; 3...dilution of antigen; 4...control; 5...serum; 6...complement; 7...R. prowazeki serial No.; 8...prowazeki; 9...the same; 10...Burneti; 11...the same; 12...without serum.

From Table 34, it can be seen that the antigen is good for the use in CBR, since it is specific, does not have anticomplementary properties, and contains 4 antigenic units:---inhibition of hemolysis ( $3/4$ ) in a 1:4 dilution (for each unit of antigen the highest dilution is used which inhibits hemolysis with the specific immune serum at  $3/4$  or  $4/4$ ).

The antigen should be considered not suitable for use in CBR if it firstly contains less than 2 antigenic units (for instance, it does not inhibit hemolysis with a specific immune serum even in a 1:2 dilution), secondly, if it is non-specific, i.e., if it inhibits hemolysis with non-specific immune sera, and, thirdly, if it has anticomplementary properties, i.e., it inhibits hemolysis in absence of immune sera.

3. The complement. --- The source of complement for CBR is the serum of guinea pigs. Blood is taken from those animals on the eve of setting up the reaction, since the complement quickly loses its activity (in 1-2 days). For getting the complement, blood is taken from the animal by means of heart puncture. In the cardiac area the hair is cut off; the skin is rubbed with alcohol, and smeared with iodine (tincture of). With the finger of the left hand the heart impulse is felt about, and at this place a puncture is carefully made with the needle which is attached to the syringe (syringe and needle should be boiled and washed thoroughly with physiological saline). The needle should be slowly and gently pressed inward toward the cardiac line. With careful movement of the needle, first a sensation of the heart pulsation is obtained, then the sensation of some obstacle from the passage through the muscular layer of the cardiac wall. By passing through the muscular layer, the needle drops into free space in the cavity of the left ventricle, and at this moment blood penetrates through the needle and the syringe, gently elevating the piston. Then the position of the syringe and needle is fixed with the left hand, and with the right hand the piston is carefully pulled out from the syringe which even under the pressure of the blood is moved upwards by itself, if the puncture was correctly made, i.e., into the cavity of the left ventricle. Not more than 5-10 ml blood can be taken from the guinea pig. After blood taking, the same volume of physiological saline should be given to guinea pig subcutaneously. The withdrawn blood is poured into a test tube which is left for  $\frac{1}{2}$  to 1 hour at room temperature or in the thermostat. The coagulated blood is encircled with a loop or a Pasteur pipet for the detachment of the bloodclot from the wall of the test tube and placed in the refrigerator for the next day.

The transparent serum, separated from the blood clot, is also the complement.

The complement taken from several guinea pigs are combined together. To avoid the exhaustion of donor guinea pigs, blood should be taken from them not more often than once in two weeks. In case of necessity, preserved complement can be also used. The preservation of complement can be done in the following manner.

The fresh complement (i.e., the guinea-pig serum) can be frozen in a special refrigerator at  $-25^{\circ}$  -  $-60^{\circ}$  temperature. Preservation of the complement can be done by way of freezing in an evaporator of an ordinary refrigerator. In the frozen shape, the complement can be kept for a long time.

Vacuum exsiccation in a volume of 0.5 - 1 ml preserves the activity of complement for many months.

The complement can be preserved by the method of GINZBURG-KALININA by way of adding 0.05 g sodium sulfate ( $\text{Na}_2\text{SO}_4$ ) and 0.04 g boric acid ( $\text{H}_2\text{BO}_3$ ) to 1 ml of guinea pig serum. Both fresh and preserved complement should be obligatorily titrated each time before setting up the complement binding reaction (see below).

4. Sheep red cells. --- For getting red cells, blood is taken from the jugular vein of sheep with the aid of a needle, directly into a sterile jar, provided with a ground-in stopper, in which glass beads are. Immediately after blood taking, the jar with the beads is shaken for 10 minutes to get defibrinated blood.

The withdrawn blood is put in a refrigerator, and in this shape it can be used for a few days.

On the day of setting up the CBR, the defibrinated blood is filtered through four layers of gauze into a centrifugal glass (or testtubes) and centrifuged at 1500-2500 r.p.m. until the sedimentation of red cells (usually 10-15 minutes). The supernatant fluid (serum) is thrown away, and the sediment of red cells is rinsed with physiological saline solution, thoroughly mixed, and again centrifuged. The washing of the red cells is repeated twice, if the supernatant fluid is transparent after the last centrifugation and is not reddened. If there is a trace of hemolysis, then the washing of the red cells should be repeated until getting a transparent and non-red-stained supernatant fluid. After the last centrifugation, the supernatant fluid is sucked off, and the sediment of red cells is used in CBR in the form of a 3% suspension in physiological saline solution (e.g., 3 ml of the red cell sediment / 97 ml of physiological saline). The red cells are washed obligatorily on the day of arranging the test. The remnant of non-used washed red cells is poured up with physiological saline solution. They can be kept for a few days in the refrigerator, and before setting up a test they are again washed.

For a longer preservation of red cells the following methods of their preservation can be used.

To 100 ml of defibrinated sheep blood, 15 ml of the following solution is added:

Physiological saline	100 ml
Boric acid	4 g
Glucose	6 g.

The bottle with the solution is boiled in water bath for 20 minutes on 3 successive days. By the indicated method the red cells can be preserved in the refrigerator for 3-4 months.

The preservation of red cells can be also done by way of direct combination of the taken sheep blood with a preservative agent of the following composition:

Sodium chloride	4.2 g
Glucose	20.5 g
Sodium citrate	8 g
Distilled water	1000 ml.



The solution should have a pH=6.2 (in case of need, it can be acidified with citric acid). The solution is sterilized in vapor steam, or boiled in the water bath for 30 minutes on three successive days.

For the preservation of red cells, sheep blood is taken in bottles which are previously rinsed out with this preserving agent (blood and the preserving agent should be in equal volumes).

The sheep blood which is preserved by one or another kind of method is kept in refrigerator, and on the day of setting up the test a triple washing of the red cells is carried out (as indicated above).

Signs of a change in the properties of red cells which make them unsuitable for the reaction are the appearance of dark discoloration and inclination to non-specific hemolysis which is manifested in reddening of the physiological solution after repeatedly rinsing the red cells, and also the lability of red cells in the reaction of hemolysis both at complement titration and at setting up the main test. At its titration the dose of complement becomes very small, and in the main test the positive sera can also give negative or weakly positive reactions.

To avoid a reduction in red-cell resistance, a regular regime should be kept in taking sheep blood: --- the blood letting (about 50-100 ml) in the same animal should not be made more often than once in 15 days. The blood is taken alternately from the right and the left jugular vein. After 1-2 years, the animals which served for the obtaining of red cells are exchanged with new ones.

5. The hemolytic serum. --- The hemolytic serum is obtained from rabbits which were immunized with sheep red cells, and the sera are issued in a ready form with the indication of their titres on the label of the ampul. The obtained hemolytic serum is transferred from the ampule into sterile test tubes, completely closed with a rubber stopper, and kept in the refrigerator. The titre of the hemolytic serum is verified only when a new batch of hemolytic serum is obtained, or when using this serum. Hemolytic serum is not titrated each time when setting up the reaction.

The determination of the titre of hemolytic serum is made in the following way. A main dilution of the hemolytic serum is prepared as 1:100 (0.1 ml of hemolytic serum / 9.9 ml of physiological saline), from which further dilutions are prepared depending upon the titre indicated on the label. (Table 35).

OUTLINE OF PREPARATION OF DILUTIONS OF THE HEMOLYTIC SERUM

Таблица 35

Схема приготовления разведений гемолитической сыворотки

№ пробирки	1	2	3	4	5	6	7	8	9
1. Разведение гемолитической сыворотки . . . . .	1:600	1:900	1:1200	1:1500	1:1800	1:2100	1:2400	1:2700	1:3000
2. Гемолитическая сыворотка 1:100 . . . . .	0,1	0,1	0,1	0,1	0,1	0,1	0,1	0,1	0,1
3. Физиологический раствор	0,5	0,5	1,1	1,4	1,7	2,0	2,3	2,6	2,9

TABLE 35 - 1...Number of test tube; 2...dilution of hemolytic serum; 3...hemolytic serum 1:100; 4...physiological solution.

EXAMPLE OF TITRATING THE HEMOLYTIC SERUM

Таблица 36

Пример титрования гемолитической сыворотки

№ пробирки	1	2	3	4	5	6	7	8	9	10
1. Гемолитическая сыворотка в разведении . . . . .	0,25	0,25	0,25	0,25	0,25	0,25	0,25	0,25	0,25	0,25
2. 3% взвесь эритроцитов	1:600	1:900	1:1200	1:1500	1:1800	1:2100	1:2400	1:2700	1:3000	0,25
3. Комплемент в разведении 1:10	0,25	0,25	0,25	0,25	0,25	0,25	0,25	0,25	0,25	—
4. Физиологический раствор	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	1,0
5. Результат	—	—	—	1+	2+	3+	4+	4+	4+	4+

TABLE 36. - 1...Number of test tube; 2...hemolytic serum in dilution; 3...3% suspension of red cells; 4...complement in 1:10 dilution; 5...physiological saline; 6...result.

In a set of test tubes directly into each, we measure out 0.25 ml of the obtained dilutions of hemolytic serum, starting with 1:600. Then, into each test tube we put 0.25 ml of a 3% suspension of washed red cells, 0.25 ml of a 1:10 dilution of complement (0.5 ml guinea-pig serum / 4.5 ml physiological saline), and 0.5 ml of physiological saline so that the total volume of the mixture is 1.25 ml. Each dilution of the hemolytic serum should be measured each time with a separate pipet; however, one pipet can be also used if the measuring is done in the reversed order, i.e., beginning with the last test tube that contains the maximum dilution of the serum.

After a thorough shaking of the mixture, the test tubes are put in a thermostat at 37° for 1 hour, and then the results are read at once, (Table 36).

The titre of the hemolytic serum is its maximum dilution which is able to hemolyze in a certain volume (0.25 ml), i.e., to solve red cells in the same (0.25 ml) volume of a 3% suspension in presence of the complement.

In the quoted example, the dilution of 1:1200 should be taken as the titre of the hemolytic serum. At setting up the complement binding reaction, the hemolytic serum is used in a triple titre, i.e., in the given case the hemolytic serum is in the dilution of 1:400 (0.1 hemolytic serum / 39.9 physiological saline solution).

### 3. ORDER OF SETTING UP THE COMPLEMENT BINDING REACTION

On the eve of setting up the main CBR test, the following preliminary work is done:

- a) separation of the sera from the blood clots; these sera will be tested (the separated could be done earlier);
- b) inactivation of the test sera in water bath at 56° for 30 minutes;
- c) taking blood from guinea pigs for getting complement;
- d) taking blood from sheep;

On the day of making the main test of CBR the following steps are taken:

- a) washing the sheep red cells (see above);
- b) complement titration;
- c) setting up the main test.

On the day of setting up the main test, work should start with washing the red cells (see above).

After the red cells were washed, the titration of the complement can start.

#### 1) TITRATION OF THE COMPLEMENT

From the test tube containing the blood taken from guinea-pigs the day before, the serum is drained off without red cells, and transferred into

a clean tube. If the blood was taken from several guinea pigs (depending upon the consumed amount of complement) then the serum of all guinea-pigs is combined. If the complement contains red cells, then a light and careful centrifugation (5 minutes at 1500 r.p.m.) should be done for the removal of red cells.

The complement is always kept on ice (or in refrigerator). The titration of the complement begins with the preparation of a hemolytic system which consists of a mixture of equal volumes of a 3% suspension of red cells and hemolytic serum in a dilution which corresponds to its triple titre. For this purpose, 9.7 ml of physiological saline and 0.3 ml of sediment of washed sheep red cells is poured in a flask. In another flask, 10 ml of physiological saline and 0.025 ml (measured with a micropipet) hemolytic serum (of the titre of 1:1200) is poured, which gives a dilution of 1:400. After thorough mixing, the red cells are poured over into the flask of hemolytic serum and, for a better mixing the mixture is thrice poured over from flask to flask and then it is put in a thermostat at 37° for 30 minutes.

#### EXAMPLE OF COMPLEMENT TITRATION

Таблица 37

Пример титрования комплемента

№ пробирки	1	2	3	4	5	6	7	8	9	10	11
2. Комплемент в разведении 1:10	0,02	0,03	0,04	0,05	0,06	0,07	0,08	0,09	0,1	0,11	0,12
3. Физиологический раствор	0,73	0,72	0,71	0,7	0,69	0,68	0,67	0,66	0,65	0,64	0,63
4. Гемолитическая система	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5
5. Результат	4+	3+	2+	1+	—	—	—	—	—	—	—

TABLE 37. - 1...Number of test tube; 2...complement in dilution 1:10; 3...physiological solution; 4...hemolytic system; 5...result.

During this time, the complement dilutions are prepared. Starting with the main dilution, we make a 1:10 dilution of the complement (0.3 ml complement / 2.7 ml of physiological saline), which is poured with micropipet into a set of dry test tubes in doses from 0.02 ml to 0.12 ml.<sup>1)</sup> (Table 37). Then, to each test tube, physiological saline solution is added up to a total volume of 0.75 ml, and then to each tube 0.5 ml of the hemolytic system is added which was kept in the thermostat for 30 minutes.

The test tubes are shaken and placed in the thermostat for 30 minutes, after which the results are read without delay.

1) FOOTNOTE: Each dose of complement is taken separately in the pipet and it is blown out into a dry test tube.

One unit of complement is the smallest amount of complement which causes full hemolysis. In the given example, one unit of complement corresponds to 0.06 ml of the 1:10 complement dilution. One full unit of complement is the (next) second test tube of full hemolysis, i.e., in the given instance, 0.07 ml complement of the 1:10 complement dilution, or 0.007 ml of the undiluted complement. A working dose of the complement is that dose which is used in the test, i.e., in case of cold binding 2 full units, and in case of warm binding, one full unit of complement.

For setting up the complement binding reaction by the cold method, in the main test two full complement units are taken, i.e., in the given instance  $0.007 \text{ ml} \times 2 = 0.014 \text{ ml}$  undiluted complement (for each test tube). If the binding is done by the warm method (i.e., at temperature  $37^{\circ}$ ), then the complement is taken in the amount of one full unit, i.e., 0.007 ml of the undiluted complement per each test tube.

Let us now give an example of calculating the necessary amount of complement for the main test at cold binding.

In the test, 100 test tubes are all together. Consequently, the undiluted complement should be  $0.014 \text{ ml} \times 100 = 1.4 \text{ ml}$ .

The needed amount of physiological saline solution (so that in a volume of 0.25 ml there should be 0.014 ml complement) consists of 0.25 ml - 0.014 ml = 0.236 ml physiological saline solution per one test tube, and for 100 tubes  $0.236 \text{ ml} \times 100 = 23.6 \text{ ml}$  physiological saline solution.

If the binding is conducted by the cold method, then the corresponding ratios of the necessary amount of complement are the following for 100 test tubes for the main test.

Complement undiluted  $0.007 \text{ ml} \times 100 = 0.7 \text{ ml}$ , physiological saline:  
 $0.25 \text{ ml} - 0.007 \text{ ml} = 0.243 \text{ ml}$ ;  $0.243 \text{ ml} \times 100 = 24.3 \text{ ml}$ .

Complement for the main test is diluted directly before its addition.

## 2) SETTING UP THE MAIN TEST.

Dilution of the sera. --- The test tubes are disposed on the stand and on each of them the supposed serum dilution is inscribed. On the first test tube the inscription includes the family name of the patient, or the order number by the record log. In the same stand are set up the test tubes for the control of serum, of antigen and of complement.

Sera activated the previous night are diluted with physiological saline in separate sets of test tubes and 0.25 ml of each dilution is placed into the test set of tubes.

The selection of the necessary dilutions of serum is made according to the problem setting. Thus, in case of the examination of the serum of a sick person, a series of dilutions is made from 1:10 to 1:2560 and even sometimes higher. But in case of mass examinations, or at the examination of people who had contact with IF patients, the CBR is set up first with a serum in 2-3 dilutions (e.g., 1:10; 1:20; 1:40), and in the presence of positive sera the latter are again examined, but already with a wider range of dilutions for the final establishment of their titre.

In conformity with the tasks of the TF serodiagnostics, in a patient the serum dilutions of sera are made 1:10, 1:20, 1:40, 1:80, 1:160, 1:320, 1:640, 1:1280, 1:2560. These dilutions are prepared in the following manner.

In the first tube, we pour 1.8 ml physiological saline, and in each of the rest of tubes 1 ml physiological saline. Then, using a separate pipet for each serum, 0.2 ml of the tested serum is taken, and transferred into the first tube where the dilution of the serum will be now 1:10. Then, after thorough mixing (three times pulling up into the pipet and blowing out), 0.25 ml of the 1:10 serum dilution is transferred into the first tube of the test set, 0.25 ml into a separate clean tube which is set up at the end of the test set of dilutions as the serum control, and 1 ml is transferred into the second tube of the set of dilutions in which 1 ml of physiological saline was poured. After thorough mixture, 0.25 ml of the obtained dilution 1:20 is transferred into the second tube of the test set, and 1 ml of the same dilution is put into the third tube of the set of dilutions, and so on, to the last dilution. Thus, we get a series of consecutive double dilutions of the serum in the test set of tubes each of which contains 0.25 ml of each of these dilutions.

The addition of antigen. --- The dry antigen of *R. prowazeki* is dissolved in physiological saline in the same volume which is indicated on the ampul's label, and after complete solution (better to dissolve somewhat earlier) 0.25 ml is added to each of the diluted sera of the test set of tubes (antigen is not added to the tube of the serum control, but in its place 0.25 ml of physiological saline solution is added). Simultaneously, a control is set up for the antigen--- in a separate test tube, 0.25 ml antigen and 0.25 ml of physiological saline solution is poured, for the verification of the absence of anticomplementary properties in the antigen.

EXAMPLE OF THE OUTLINE OF A CBR SET-UP

Таблица 38

Пример схемы постановки РСК

1 Ингредиент	2 Разведение сыворотки						3 Контроль сыворотки	4 Контроль антигена	5 Контроль комплемента			6 Контроль гемолитической системы
	1:20	1:40	1:80	1:160	1:320	1:640			2	1	0,5	
7 Сыворотка	0,25	0,25	0,25	0,25	0,25	0,25	0,25	—	—	—	—	—
8 Антиген	0,25	0,25	0,25	0,25	0,25	0,25	—	0,25	—	—	—	—
9 Комплемент	0,25	0,25	0,25	0,25	0,25	0,25	0,25	0,25	0,25	0,12	0,06	—
10 Физиологический раствор	—	—	—	—	—	—	0,25	0,25	0,5	0,63	0,69	—
11 В рефрижератор при 4° на 18—20 часов (или при 37° на 1 час)												
12 Гемолитическая система	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5
13 Физиологический раствор	—	—	—	—	—	—	—	—	—	—	—	0,7
14 В термостат при 37° на 30 минут												
15 Ожидаемый результат в контролях							—	—	—	—	2+	4+

TABLE 38. - 1...ingredient; 2...serum dilutions; 3...serum control; 4...antigen control; 5...complement control; 6...control of the hemolytic system; 7...serum; 8...antigen; 9...complement; 10...physiological saline solution; 11...in the refrigerator at 4° for 18-20 hours (or at 37° for 1 hour); 12...hemolytic system; 13...physiological solution; 14...In thermostat at 37° for 30 minutes; 15...Expected results in the controls.

The addition of complement. ---In accordance with the calculation made at the titration (see above), the complement is diluted in the required amount with physiological saline solution and at once it is added in 0.25 ml amounts (the so-called working dose) to each test tube of the diluted serum and with the antigen added to it. Complement is also added to the serum control and antigen control tubes.

Simultaneously, a control is set up for the complement. If the binding is conducted at 37°, then one complement control is set up with its working dose, i.e., in a separate tube 0.25 ml of complement is poured in the test tube (one working dose), and instead of serum and antigen, 0.5 ml of physiological saline is added. If the binding reaction is made at 4°, then the complement is set up in three doses for the calculation of the actual amount of doses of complement taken in the test. For this, three test tubes are taken. The first tube is marked with figure 2, the second with figure 1 and the third with figure  $\frac{1}{2}$ , correspondingly to two full units of complement (used in the test for binding in the cold), to twice less and

to 4 times less number of complement units. In the first tube with the mark 2, 0.25 ml complement is poured, which is poured in all tubes of the test set (two full units of complement). In the second tube with the mark 1, with a clean dry micropipet, 0.12 ml of the same complement is poured (one full unit of complement), and in the third tube (marked  $\frac{1}{2}$ ), with the same micropipet, 0.06 ml of the same complement is poured ( $\frac{1}{2}$  of one full unit of complement). Then, into each of the three tubes, physiological saline solution is added up to a total volume of 0.75 ml, i.e., into the tube marked 2, 0.5 ml saline is added, into the tube marked 1, 0.63 ml of saline is added, and into the tube marked  $\frac{1}{2}$ , 0.69 saline is added (Table 38). The stand with the test tubes is placed in the refrigerator for 18-20 hours (or in the thermostat at 37° for 1 hour).

The addition of the hemolytic system. --- After 18-20 hours, if the binding is conducted in the cold, or after an hour stay of the stand with the tubes in the thermostat, the hemolytic system is added which, as already indicated, consists of a mixture of equal volumes of a 3% suspension of red cells and dilution of the hemolytic serum, corresponding to its triple titre. For instance, if there are 150 tubes altogether in the test, and the titre of the hemolytic serum is 1:1200, then first 38.8 ml physiological saline solution is poured in a flask, and 1.2 ml of erythrocytes is added, and in a second flask 39.9 ml physiological saline solution is poured and 0.1 ml hemolytic serum is added so that its dilution will be 1:400. Then, the suspension of red cells is poured into the flask with the diluted hemolytic serum, and after a triple pouring from one flask into the other, the mixture is placed in the thermostat at 37° for 30 minutes. At the same time, from the refrigerator the test stand with the tubes is taken out if the binding is conducted in the cold.

After 30 minutes, in 0.5 ml amounts the hemolytic system is added to each test tube, and a control is set up for the hemolytic system, which should show complete absence of hemolysis. For this purpose, in a separate tube, 0.5 ml of the hemolytic system and 0.75 ml of physiological saline solution (in place of serum, antigen, and complement) is poured. After addition of the hemolytic system, each test tube is thoroughly shaken so that no sedimented red cells should be at the bottom of the tube. The stand with the test tubes is put into the thermostat at 37° for 30 minutes (the time of stay can be somewhat shortened or lengthened depending upon the completion of the hemolysis in the serum, antigen and complement controls.)

The reading of results is done one hour after the test tubes were taken out from the thermostat. The results of the reaction are marked with crosses (plus signs):

4/...absence of hemolysis; the fluid above the sediment of red cells is transparent and not reddened;

3/...traces of hemolysis; the fluid above the sediment of red cells is slightly reddened in a rosy color;

2/...plain hemolysis; the fluid above the sediment of red cells is of red color. There is considerable sediment of red cells at the bottom of the test tube;

1/...almost complete hemolysis. Very slight sediment of red cells at the bottom of the test tube.

- ...complete hemolysis; there is no red-cell sediment.



Each test should be combined with setting up the reaction with an established positive serum of known titre so that there should be a continuity in tests. The titre of the known serum (better to use dry standard serum) should be checked.

The set-up of the reaction can be considered reliable if all the established arranged controls confirmed the correctness of the reaction course, i.e., if in the controls of the tested sera, in the antigen control and in the complement control (working dose) there is complete hemolysis. And, in the control of the hemolytic system, the hemolysis is absent. In the complement control, with setting up the binding in the cold, in the two first test tubes there should be full hemolysis (2 and one full unit of complement), and in the third tube ( $\frac{1}{2}$  a unit) the inhibition of hemolysis should be  $\frac{2}{3}$  or  $\frac{3}{4}$ .

If in the control of any of the tests sera even a slight inhibition of hemolysis is noted, then the reaction with this serum cannot be considered at all, and in the record log the notation should be made about its anti-complementarity.

If in the antigen control, there is even an insignificant inhibition of hemolysis, then none of the tests can be read.

In excess of complement in the reaction in the third tube of complement control ( $\frac{1}{2}$  unit) a full hemolysis can be observed. In this cases the titres of the tested sera may happen to be understated. On the contrary, in case of complement shortage, absence of hemolysis can be noted even in the second tube of the complement control. In this case, the titres of the tested sera will be overestimated. In such cases, the whole test must be repeated.

The titre of a serum according to the CBR is the smallest amount of serum (or the largest dilution) which inhibits hemolysis at not less than  $\frac{2}{3}$  with markedly positive reactions ( $\frac{3}{4}$  or  $\frac{1}{2}$ ) in the preceding serum dilutions.

#### INDIRECT HEMAGGLUTINATION REACTION

The essence of the reaction of indirect hemagglutination (HAR) is that in case of the interaction of an immune serum with sheep red cells, on whose surface an antigen is absorbed that is specific for the given serum, the red cells become agglutinated, which is well visible with the naked eye.

At setting up the reaction with different successive dilutions of the tested serum, the hemagglutination titre of a certain serum can be established.

##### 1. INGREDIENTS OF "HAR" AND THEIR PREPARATION

For HAR, the following ingredients are needed:

- a) the tested serum or a definitely immune serum;
- b) antigen of R. prowazeki for HAR;
- c) sheep red cells.

- 1) The tested serum.--- The serum is prepared just in the same way

as for CBR (see above), i.e., the serum is separated without red cells from the blood clot. The blood was taken from a vein or from the finger (if necessary, it is centrifuged). Serum can be also used which was obtained after taking blood from the finger in citrated physiological saline solution.

The serum obtained by any method should be inactivated at 56° for 30 minutes.

Since the human serum may contain normal heterogeneous hemagglutinins for sheep red cells, it must be obligatorily exhausted by sheep red cells to avoid the presence of non-specific homagglutinins in the serum.

2). The antigen. --- In indirect HAR, sheep red cells are used as antigen on whose surface RPr antigen is absorbed. The latter is prepared from egg cultures of rickettsiae and dried out in vacuum.

On the label of the antigen ampul, the volume of physiological saline solution should be inscribed in which the contents of the ampul should be dissolved, which in its turn is established by preliminary titration of the antigen (in the laboratory which prepares the antigen) with the calculation that in the working dose not less than two antigenic units should be contained.

3). Sheep red cells.---Just as for CBR test (see above), the sheep red cells are prepared by the same method, i.e., the defibrinated blood is filtered through 4 layers of gauze, centrifuged, and the red cells are washed 2-3 times with physiological saline solution. After subsequent centrifugation, the supernatant transparent and colorless fluid is removed, and the sediment of red cells is used for the HAR.

## 2. ORDER OF SETTING UP THE "HAR".

On the night previous to the setting up of the reaction, the following steps are taken:

- 1) inactivation of the tested sera (it can be also inactivated earlier);
- 2) taking blood from sheep (the blood is ready for a few days);

On the day of making the reaction, the following is done:

- 1) washing the sheep red cells;
- 2) absorption of antigen by sheep red cells;
- 3) absorption of heterogeneous hemagglutinins by normal sheep red cells from the tested sera.

## 2. Arrangement of the HAR.

1. Solution of the dry antigen of R. prowazeki.---The dry RPr antigen for the HAR is dissolved in that much volume of physiological saline solution as indicated on the label of the ampul.

2. Preparation of antigen adsorbed on red cells for the setting up of the HAR. --- Four ml of dissolved RPr antigen is measured out with a pipet, and transferred into a centrifugal tube. Then, 0.1 ml sheep red cells (washed sediment of red cells) is added, and after thorough mixture, it is put into the thermostat at 37° for 1 hour, with periodical shaking of the mixture every 10-15 minutes for better adsorption of the antigen on the red-cell surface.

3. Adsorption of the heterogenous hemagglutinins from the tested serum. --- For the adsorption of the heterogenous hemagglutinins the tested serum is mixed with sheep red cells (washed sediment of red cells) in a volume corresponding to half of the volume of the used serum. For instance, in the centrifugal tube 0.9 ml physiological saline and 0.1 ml tested inactivated serum is added (the obtained dilution is 1:10), and then 0.05 ml sheep red cells is added. The mixture is left at room temperature for 16-20 minutes, with periodic shaking.

4. Centrifugation of the antigen. --- The centrifugation of the antigen, i.e., of the erythrocytes which stayed for one hour in the thermostat for the adsorption of the RPr antigen by the red cells, is done for 10 minutes at 2500 r.p.m.. Then, the supernatant fluid is thoroughly and completely removed. To the sediment of red cells, 10 ml physiological saline solution is added. The mixture, which thus consists of a 1% suspension of red cells with the adsorbed rickettsial antigen, is used as antigen in the HAR.

5. Centrifugation of the tested serum. --- The centrifugation of the serum for its liberation from the added sheep red cells to adsorb the heterogenous hemagglutinins is done for 10 minutes at 2500 r.p.m.

The supernatant fluid is sucked off into a clean test tube, and the sediment of red cells is thrown away (the centrifugation of the serum can be done simultaneously with the centrifugation of the antigen).

6. Dilution of the tested sera. --- In a stand, test tubes are placed, and they are marked for the following dilutions: --1:250; 1:500; 1:1000; 1:2000; 1:4000; 1:8000; 1:16000; 1:32000 and 1:64000 (Table 39). On the first test tube, the patient's family name is indicated, or the order number according to the recording log. Then, three more test tubes are put on the stand for serum control, "antigen control and control of normal sheep red cells. All tubes are marked.

The dilution is made in a separate set of test tubes. For this, in the first tube 2.4 ml physiological saline solution is placed, and in each of the other tubes 1 ml. Then, in the first tube 0.1 ml inactivated and adsorbed test serum is added (the serum was diluted 1:10 at the adsorption by the red cells). Thus, in the first tube a dilution of 1:250 is obtained. From this dilution, into the first tube of the test set 0.4 ml is transferred, and into the second diluting tube 1 ml, with 1 ml of physiological saline solution. In the second tube the dilution thus becomes 1:500. From this dilution 0.4 ml of the mixture is again transferred into the second tube of the test set, and 1 ml into the third dilution tube with 1 ml of physiological saline solution, and so on, to the end.

OUTLINE OF SETTING UP THE "HAR"

Таблица 39

Схема постановки РГА

1 Ингредиенты	2 Разведение сыворотки								3 Контроль эритроцитов	4 Контроль антигена	5 Контроль сыворотки
	1:250	1:500	1:1000	1:2000	1:4000	1:8000	1:16 000	1:32 000			
6 Исследуемая сыворотка, мл	0,4	0,4	0,4	0,4	0,4	0,4	0,4	0,4	—	—	0,4
7 Антиген, мл (1% взвесь эритроцитов с адсорбированным антигеном)	0,1	0,1	0,1	0,1	0,1	0,1	0,1	0,1	—	0,1	—
8 Физиологический раствор, мл	—	—	—	—	—	—	—	—	0,4	0,4	—
9 1% взвесь эритроцитов барана, мл	—	—	—	—	—	—	—	—	0,1	—	0,1
10 Ожидаемый результат реакции в контролях									Отрицательный //	Отрицательный //	Отрицательный //

**TABLE 39.** - 1...ingredient; 2...serum dilution; 3...red cell control; 4...antigen control; 5...serum control; 6...tested serum ml; 7...antigen, ml (1% cell suspension with adsorbed antigen); 8...physiological saline, ml; 9...1% sheep red cells suspension, ml; 10...desired result of reaction in the controls; 11...negative.

7. Addition of the antigen. ---To each tube, 0.1 ml antigen, i.e., 1% sheep red cell suspension is added on the surface of which RPr antigen is adsorbed. Each tube is thoroughly shaken for obtaining an homogeneous suspension.

8. Control for the test. ---At the same time the following controls are set up:

a) A serum control is set up to verify that the test serum itself does not cause agglutination of sheep red cells. For this purpose, a 1% suspension of normal (without RPr antigen) sheep red cells is prepared (9.9 ml of physiological saline solution and 0.1 ml of sheep red cells). In the test tube, with corresponding marking, 0.4 ml of the test serum is poured in a 1:250 dilution (inactivated and exhausted), and 0.1 ml of a 1% suspension of sheep red cells.

b) Antigen control for the antigen used in the test (1% suspension of sheep red cells with adsorbed RPr antigen) is arranged for the purpose to check whether the antigen itself would result in red cell agglutination in the absence of the specific serum. For this purpose, in a test tube 0.1 ml antigen is poured with 0.4 ml physiological saline solution.

c) Sheep red-cells control is arranged for the purpose to check whether the normal red cells of sheep would agglutinate the red cells without antigen and without specific serum. Therefore, in a test tube 0.4 ml physiological saline solution is measured and 0.1 ml of a 1% suspension of sheep red cells is added (see above).

All test tubes are put in the thermostat at 30° for the night, or at room temperature (not lower than + 20°) for the same period.

Parallel with the test sera, a reaction should be also set up for the control of the standard serum of known titre.

9. Reading the results of the reaction.---The results of the reaction are read next day. The reading of the reaction is done with the naked eye, starting without shaking the test tubes, according to the presence or absence of red cell agglutination. In case of a positive reaction, marked with a plus sign (+), on the bottom of the test tube, there is a wide granular sediment of coagulated red cells in the shape of a upside-down umbrella. This sediment of agglutinated red cells can be spread out evenly on the bottom of the test tube, or it can make a form which resembles a ring. At shaking, the test tube, the agglutinated red cells are separated in flakes from the bottom of the tube, sometimes with difficulty. In case of a negative reaction, i.e., in case of absence of agglutination of the red cells, the latter will sediment in the center of the test tube's bottom, forming an even small disk with smooth edges. At shaking the test tube, the red cells rise up from the bottom of the test tube in the form of a delicate stream and form a homogenous suspension.

The reaction can be considered reliable if:

a) in absence of antigen the tested serum does not cause agglutination of normal sheep red cells;

b) the antigen itself, i.e., the 1% suspension of sheep red cells with the adsorbed antigen, does not cause agglutination of the red cells in the absence of serum;

c) the normal sheep red cells do not give spontaneous agglutination, i.e., in the red-cell control the reaction is negative:

If in the controls, agglutination of red cells occurs, no reaction can be considered.

In a given setup of reaction, the titre of the control immune serum should correspond with its known titre.

#### RICKETTSIAL AGGLUTINATION REACTION (RAR)

The rickettsial agglutination reaction (RAR) is made in thoroughly washed and thoroughly sterilized test tubes.

The test serum should be freshly taken, without red cells, and non-hemolyzed. Serum dried on paper is not suitable for the setting up of an RAR.

The serum should not be inactivated.

The antigen which is used in the agglutination reaction for the diagnosis of TF consists of a suspension of *Rickettsiae prowazeki* cultivated in the vitelline sacs of chick embryos, or accumulated in the lungs of intranasally infected mice. The concentration of antigen should correspond to the turbidity of bacterial optical standard with 500 million microbic bodies in 1 ml.

The antigen, prepared in the dry form (vacuum exsiccation in saccharose) should be quickly dissolved, and the obtained suspension should be homogenous, without flakes.

The antigen is dissolved before use in the volume of physiological saline solution which is indicated on the label of the ampul.

1. SETTING UP THE RICKETTSIAL AGGLUTINATION REACTION BY THE VOLUMETRIC METHOD.

The tested serum is diluted with physiological saline solution at such a rate that successive double dilutions are obtained, e.g., 1:10; 1:20; 1:40; 1:80; 1:160; 1:320; 1:640; 1:1280. It should be considered here that after the addition of an equal volume of antigen the dilutions of serum are doubled, and, consequently, the above indicated dilutions will actually correspond to 1:20, 1:40; 1:80; 1:160; 1:320; 1:640; 1:1280; 1:2560. In correspondence with this dilution, we should mark the test tubes.

The dilution is made in a separate series of test tubes: in the first tube, 1.8 ml physiological saline is put, and in each of the other tubes 1 ml. Then, to the first tube we add 0.2 ml test serum, and we get a dilution of 1:10. From this first tube, after mixing (three times pulling up and blowing out the fluid), with a graduated pipet we take 0.2 ml and transfer it into the first tube of the test set on which the mark reads 1:20 dilution, then 0.2 ml of the same dilution of serum is measured out into a separate tube (the serum control) and 1 ml into the second tube of the dilution set which already has 1 ml physiological saline solution. After mixture, 0.2 ml is transferred into the second tube of the test set whose the mark reads 1:40 dilution, and 1 ml into the third dilution tube with 1 ml of physiological solution, and so on.

The serum can be diluted also in the test set of tubes. In this case the dilutions are made in the following form: --- into each of the set of agglutination tubes, with the exclusion of the first tube, 0.2 ml of physiological solution is poured. Then, in a separate tube a 1:10 dilution of the serum is prepared (0.1 ml of serum and 0.9 ml of physiological solution), which after mixing is added in 0.2 ml volume to the first test set of tubes (on which the mark is 1:20 dilution), then into the second tube (where the mark is 1:40 dilution), and 0.2 ml into a separate tube (the serum control). From the second test tube (marked 1:40 dilution) 0.2 ml is transferred after mixing into the third tube, mixed, and 0.2 ml is again transferred into the fourth tube, and so on. From the last tube in which the volume happens to be 0.4 ml, 0.2 ml is taken out and discarded.

To the diluted 0.2 ml amount of the serum, whichever way the dilution was made, 0.2 ml antigen is added to each tube. Parallel with this, an antigen control is set up: --- 0.2 ml antigen + 0.2 ml physiological saline solution. To the serum control tube, 0.2 ml physiological saline is added.

After shaking the test tubes, they are put into the thermostat at

37° for 18-20 hours. The reading of results is done on the next day two hours after the tubes were taken out from the thermostat. At the estimation of results, it should be considered that in difference from the agglutination of bacteria, the agglutination of rickettsiae is very delicate, and the formed flakes are very easily rocked and destroyed. Therefore, the stands with the tubes should be carefully taken out from the thermostat, and at reviewing the reactions, the tubes should not be shaken in any case.

The reading of results is done with the naked eyes (without a magnifying glass) according to the following outline:

4+...complete clearing of the fluid above the sediment in presence of a granular sediment at the bottom of the test tube, arranged in the form of a capsized umbel;

3+...no complete clearing of the fluid above the sediment, sediment similar to the one in the 4+ reaction;

2+...turbid fluid in presence of a slight granular sediment at the bottom and wall of the test tube;

1+...slight granularity of the suspension.

- ...negative reaction (homogenous suspension, no difference from the suspension in the antigen control tube).

The result is considered reliable if in the antigen control the rickettsial suspension remains homogenous, and in the serum control tube the fluid was transparent and no flakes appeared.

The reaction is considered positive in the presence of intensive agglutination of rickettsiae, marked with not less than 2+ in the final dilution, and in presence of a reaction of 3+ and 4+ in the initial dilutions of the serum.

Parallel with the tested sera, a reaction should be also set up to control the agglutination with an established positive serum of known titre with the same RPr antigen.

In the absence of graduated pipets, the RAR can be also set up in the drop method. Here, it should be considered that 0.1 ml corresponds to 2 drops.

## 2. SETTING UP THE RAR WITH THE DROP METHOD ACCORDING TO MOSING.

For the purpose of saving antigen, for the diagnosis of TF, the RAR can be made by the drop method of MOSING.

For arranging this reaction, a special RPr antigen is used where the rickettsiae were accumulated in the intestines of infected lice. This reaction can be also successfully set up with the usual dry antigen of RPr for agglutination in the usual concentration, i.e., 500 million microbic bodies per 1 ml.

The difference in the reactions with these antigens will be that the antigen from infected lice has a cinnamon color, while the antigen from the egg cultures of rickettsiae is colorless.

It should be considered that the drop agglutination according to MOSING gives usually 2-4 times lower titres for the sera than the ordinary agglutination, and for this reason its diagnostic titre is lower (1:40). The reaction is entirely specific.

The setup of the reaction is done in the following manner. In small test tubes of 0.8 - 1 ml volume a series of dilutions are made of the tested serum with physiological saline by the drop method. In the first tube, 4 drops of saline are placed, in the subsequent tubes 3 drops each. Then, to the first test tube, a drop of serum is added, i.e., a dilution of 1:5 is obtained. After mixing, from this dilution 3 drops are transferred into the second test tube, obtaining therewith a 1:10 dilution. Then, 3 drops of the second tube are transferred into the third, and 3 drops of the third tube into the fourth, and so on.

Thus, a series of dilutions is obtained: ---in the first test tube the dilution is 1:5 in a volume of 2 drops, and in the subsequent test tubes the dilutions are 1:10, 1:20, 1:40, 1:80, 1:160, 1:320, in a volume of 3 drops. To this dilution of serum, an equal amount of antigen is added. Thus, the serum in each of the tubes is still doubly diluted, i.e., actually the serum dilutions are 1:10, 1:20, 1:40, 1:80, 1:160, 1:320, 1:640.

For the serum control, it is diluted with physiological saline solution to 1:10 (one drop of serum + 9 drops of physiological saline).

For the antigen control, 3 drops of antigen are mixed with an equal amount of physiological saline. To prevent the contents of the tubes from exsiccation, it is recommended that each tube be plugged with a cotton plug.

The stands with the series of test tubes are shaken and placed in the thermostat at 30° temperature for 20-24 hours. The reading is done on the next day not earlier than 2-3 hours after the tubes were taken out from the thermostat. In case of a positive reaction, a dark brown sediment is obtained in the shape of an umbel with irregular edges, with transparent supernatant fluid.

In case of a negative result, sediment is not observed, or it is collected in the center of the test tube bottom, and the supernatant fluid is turbid. The nature of the agglutination of rickettsiae is finely granular.

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